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Maxadilan

CLONING AND FUNCTIONAL EXPRESSION OF THE GENE ENCODING THIS POTENT VASODILATOR PEPTIDE*

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Maxadilan is a potent vasodilator peptide released into the skin when the sand fly *Lutzomyia longipalpis*, an important vector of leishmania, probes for a blood meal. As several lines of evidence suggest that this peptide may play a critical role in the enhancement of leishmania infectivity attributed to sand fly saliva, the peptide has been proposed as a candidate antigen for a leishmanial vaccine. Although maxadilan is the most potent vasodilator peptide known and shares several properties with calcitonin gene-related peptide (CGRP), studies of its structure, physiological effects, and biological roles have been limited by the miniscule quantities available. Here we report the isolation of cDNA and genomic DNA clones that encode maxadilan. The predicted translation product shows no significant homology with any previously isolated proteins. The coding DNA has been expressed in *Escherichia coli* and the purified recombinant peptide is biologically active with a specific activity comparable to the natural peptide. Recombinant maxadilan will be useful in studies of vascular biology and could lead to novel therapeutic and prophylactic agents.

The saliva of hematophagous insects, such as sand flies or mosquitoes, contains a variety of substances which aid in obtaining a blood meal (1). These secretions contain antiserotonin, antithromboxane, and various prostaglandin components which prevent vessel contraction and other components which prevent platelet aggregation. The high potency of these substances suggests that they could be used as probes for specific host receptors, as tools to study the physiology of vascular and hematologic processes, and perhaps as novel therapeutic agents. A limitation in the study and potential application of these salivary gland components is the fact that they are present in minute quantities.

The bite of the New World sand fly *Lutzpmyia longipalpis* results in an irregular red spot surrounded by pallor not associated with itching or pain. The erythema is caused by a potent vasodilator peptide named maxadilan (2). Injection of less than 100 pg of natural maxadilan into rabbit skin produces erythema which persists for hours, making it the most potent vasodilator peptide characterized to date. The response to maxadilan is very similar to that seen for calcitonin gene-related peptide (CGRP)[1] and maxadilan and CGRP reportedly share chromatographic, immunologic, and pharmacologic properties (3). However, on a molar basis, maxadilan has at least 100-fold greater potency than CGRP as an erythema-inducing agent. The mechanism of action of maxadilan is not known. A likely possibility is that it acts as an agonist on the receptor for CGRP although other mechanisms, such as acting as an antagonist on the receptor for the vasoconstrictor peptide endothelin, are possible.

Several of the more than 600 species of sand flies are important to man since they serve as the vector of *Leishmania*, the protozoan parasite which causes the disease leishmaniasis. During the course of a blood meal the sand fly salivates into the skin of its vertebrate host, transmitting *Leishmania* if the fly is infected. Sand fly saliva is critically important to the infectivity of *Leishmania* subsequent to injection of the small number of parasites normally transmitted during sand fly feeding (4). Recent studies have shown that the enhancement of *Leishmania* infection by saliva can be mimicked by CGRP (5), thereby suggesting that maxadilan may play an important role in the enhancement. Salivary gland extracts have also been shown to have direct effects on macrophages and thus may be responsible for the increased infectivity of *Leishmania*. These effects include the prevention of γ-interferon activation and the inhibition of antigen presentation (6). The fact that CGRP also mimics these effects further implicates maxadilan in the enhanced *Leishmania* infectivity. Protection from *Leishmania* might be achieved if the "enhancement factor" could be neutralized by a host antibody, and it has been suggested that this factor is a potential vaccine target (5).

Studies on the mechanism, biological roles, and vaccine potential of maxadilan require greater amounts of protein than is practically available from natural sources. Toward that goal, we have obtained and characterized cDNA and genomic clones encoding maxadilan, expressed the coding DNA within *Escherichia coli* and recovered a biologically active peptide having a specific activity comparable to the natural peptide.

MATERIALS AND METHODS

Isolation and Amplification of cDNA—The procedure used to prepare cDNA was a modification of the method of Belyavsky *et al.* (7). RNA was extracted from 10 pairs of dissected sand fly salivary glands and reverse transcribed with avian myeloblastosis virus-reverse transcriptase (Life Sciences) using the primer 5′ GGGAGGCCCCT

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) M77090.

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[1] The abbreviations used are: CGRP, calcitonin gene-related peptide; PCR, polymerase chain reaction; 3′-UT, 3′-untranslated; HPLC, high performance liquid chromatography.

TTTTTTTTTTTTTTT 3′. Two % of this reaction volume (0.8 μl) was used in a 100-μl poylmerase chain reaction (PCR) containing 0.2 mM dNTPs, 0.1 mM dithiothreitol, 20 mM $(NH_4)_2SO_4$, 70 mM Tris, pH 8.65, 2 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 0.1% Triton X-100, and 100 pmol of each primer. The primer above was used in conjunction with a degenerate 32-mer primer based on the tentative amino acid sequence of residues 3–13: 5′GC(C/T)ACCGA(C/T)CA GTTCCG(C/T)AAGGC(C/T)AT(C/T)GA(C/T)GA3′. Thirty cycles were run on a Coy Temp Cycler with annealing at 50 °C for 3′30″, extension at 72 °C for 3′30″, and denaturation at 94 °C for 1′. An aliquot of the PCR mixture was treated with the Klenow fragment of DNA polymerase, run on a 3% NuSieve GTG (FMC) agarose gel, subcloned into the *Hinc*II site of Mp18, and sequenced.

Cloning of the Maxadilan Gene—A genomic sand fly library was constructed in the bacteriophage vector EMBL3 (Promega) using a partial Sau3A digest of DNA extracted from a *L. longipalpis* sand fly egg cell line (8) derived from flies of a different isolate than the flies used for the amino acid sequencing and cDNA experiments. This library was screened with amplified cDNA radiolabeled with ^{32}P by random priming and four strongly hybridizing clones were obtained. A 600-base pair region from one of these clones was amplified with primers based on the EMBL 3 polylinker site and nucleotides 407–386 of the maxadilan cDNA clone. The amplified DNA fragment was sequenced upstream of the maxadilan-coding DNA. Based on this sequence, a primer, 5′ CCCGGATCCGACCTATTAACCAGAAAG 3′, was prepared that matched sequence upstream of the sequence depicted in Fig. 2. This primer was used in conjunction with a primer complementary to 3′-untranslated sequences (3′-UT primer) in a PCR to amplify genomic DNA from the same isolate of flies from which the cDNA had been prepared. Both strands of this DNA fragment were then sequenced.

Expression of Recombinant Maxadilan—The complete predicted mature maxadilan coding sequence was amplified with the oligonucleotides 5′ CCGGATCCTGTGTGATGCAACATGCCAA 3′ and 5′ CCGAATTCACTTTCCTGCCTTAAATTC 3′. The resulting DNA fragment was digested with *Bam*HI and *Eco*RI and subcloned into the *Bam*HI/*Eco*RI site of the bacterial vector pGEX-3X which expresses inserted coding DNA as a fusion protein with glutathione *S*-transferase (9). The maxadilan-glutathione *S*-transferase fusion protein was expressed, cleaved with activated Factor X (Xa) (Sigma), and isolated as described (9). Expression and cleavage were followed by electrophoresis on high density PHAST gels (Pharmacia LKB Biotechnology Inc.). The supernatant containing recombinant maxadilan was further purified on a micropellicular C-18 reversed-phase HPLC column under an acetonitrile gradient (2) and lyophilized. After Xa cleavage, the recombinant maxadilan has a Gly-Ile-Leu sequence preceding the initial cysteine of the natural peptide. In addition, because the COOH-terminal PCR primer was designed based on the original genomic sequence which was obtained from a different sand fly isolate, the COOH terminus of recombinant maxadilan has Lys-Ala at positions 60–61 rather than the Ser-Ser encoded by the cDNA and gene sequence indicated in Fig. 2.

Biological Activity of Maxadilan—Maxadilan isolated and purified by HPLC from sand flies (2) or HPLC purified recombinant material was lyophilized, resuspended in water, and quantified by amino acid analysis. As an initial test of biological activity, 30 μl of various dilutions of maxadilan in phosphate-buffered saline, pH 7.4, were injected intradermally into the skin of a shaved rabbit (not shown). For the human skin experiments, 10-μl injections were given via a 30 gauge needle into the skin of one of the authors and photographed 45 min later.

RESULTS

NH_2-terminal Sequence of Maxadilan—Maxadilan has recently been isolated from dissected salivary glands by freeze-thaw lysis, purified by C-18 reversed-phase HPLC and found to have a mass of 6839d based on mass spectrometry (2). Amino-terminal microsequencing of maxadilan isolated from 100 pairs of salivary glands tentatively determined amino acid residues 3–13 as AT(D)QFRKAIDD. The first 2 residues could not be determined because of high background and the Asp predicted at position 5 was based on a very weak signal.

Cloning of the Maxadilan Coding DNA—Total RNA was prepared from 10 pairs of salivary glands and a cDNA copy was synthesized. A degenerate oligonucleotide corresponding to amino acid residues 3–13 was designed and synthesized as described under "Materials and Methods." This primer was used in conjunction with an oligo(dT) primer in the PCR to amplify a portion of the putative maxadilan cDNA. A single DNA fragment of approximately 350 base pairs was obtained in the PCR reaction (Fig. 1). The amplified DNA fragment was cloned and sequenced revealing an open reading frame encoding 50 amino acids downstream from the 3–13 primer (Fig. 2*A*).

A sand fly genomic library constructed in EMBL3 as described under "Materials and Methods" was next screened for clones homologous to the putative maxadilan cDNA. One positive clone was isolated and sequenced in the region upstream of the homology to the putative maxadilan cDNA. An oligonucleotide, identical to a sequence several hundred base pairs upstream of the 3–13 primer site, was used in conjunction with an oligonucleotide near the poly(A) addition site (3′-UT primer) to amplify a genomic fragment from the same sand fly isolate as was used to obtain the cDNA fragment. Sequence of this fragment is shown in Fig. 2*B*. The genomic DNA contains the same sequence as the PCR generated cDNA downstream of the 3–13 primer and contains no introns in this region. The sequence containing the 3–13 primer homology correctly encodes the 10 amino acids confidently predicted by the amino-terminal sequencing of maxadilan, confirming that both the cDNA and genomic clones encode the peptide purified as maxadilan. The cysteine at position 5 encoded by the cDNA corresponds to the weak aspartic acid prediction in the amino acid sequencing. Immediately upstream of the primer site, the genomic DNA encodes 18 amino acids preceded by a methionine codon and, four codons earlier, a termination codon. Curiously, this sequence does not appear to encode a secretory leader as would be expected for a secreted salivary protein. Analysis of the genomic DNA further upstream (base pairs 141–196) does reveal a sequence encoding a classical leader sequence preceded by a methionine codon and followed by a canonical RNA splice donor site. To test the possibility that this sequence represents an exon of the maxadilan gene, a PCR experiment was performed. First, four new oligonucleotides were prepared. These primers, depicted in Fig. 2*B* were: *A*, immediately 3′ to a putative TATAA signal preceding the putative exon; *B*, immedately upstream of the putative initiating ATG; *C*, within the coding sequence of the putative first exon; and *D*, within the putative intron. These four upstream primers were used in conjunction with a downstream primer, 5′ CTTTCCTGCCTTAAATTC, to

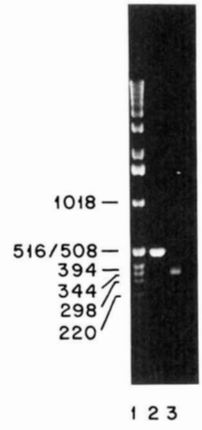

FIG. 1. **PCR amplification of partial maxadilan cDNA.** 3% Nu-Sieve GTG agarose gel (FMC Bioproducts) was run with: *lane 1*, 1000-base pair ladder (Bethesda Research Labortories); *lane 2*, 500-base pair positive control amplified band (Perkin Elmer-Cetus); *lane 3*, partial maxadilan cDNA.

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                    3-13 primer
          GCYACCGAYCAGTTCCGYAAGGCYATYGAYGACTGCCAGAAGCAGGCGCATCATAGCAAT
                                                CysGlnLysGlnAlaHisHisSerAsn

GTTTTGCAGACTTCTGTACAAACAACTGCAACATTCACATCAATGGATACCTCCCAACTACCTGGAAATAGT
ValLeuGlnThrSerValGlnThrThrAlaThrPheThrSerMetAspThrSerGlnLeuProGlyAsnSer

GTCTTCAAAGAATGTATGAAGCAGAAGAAAAAGGAATTTAGTTCAGGAAAGTAAAAGATTGAAGAAAATTGT
ValPheLysGluCysMetLysGlnLysLysLysGluPheSerSerGlyLys---

AGCCGAGGAGAGAAAGAAAGAAAGTCCCATACCATATTTTGTTTGTTAATTGTAACGAATTTTCCGAAAAAA

TAAAATATTATGCACTCAATTTAAAAAAAAAAAAAAAAAGGGGCCTCCC
                      oligo dT primer


AATCAATTGCTAAAAAAAAATTACAAATAGAACTACTACAGATGTTCTGAATTTTTTCTTGATATTCTTTCT 72

                    A              *cap?          B
CAATTGGATGTATAAAAGAGGCTATTTTGTGCTGATTTTGTTAGTCAGTATTCTGATAAACTACAAAAATGA144
                                                                    MetL
                  C                                D
AGCAAATCCTTTTAATCTCTTTGGTGGTGGTTCTTGCCGTGTTTGCCTTCAGTAAGTTCTTCCTTTAGGCCT216
ysGlnIleLeuLeuIleSerLeuValValValLeuAlaValPheAlaPheA.....................

TTCCTTCTCAAAACTTAAAGTAATTTAATGAAATATTCTTTAAATAATCTCCATTTTCTTGTAGACGTTGCT288
...........intron.....................................................snValAla

GAGGGCTGTGATGCAACATGCCAATTTCGCAAGGCCATAGATGACTGCCAGAAGCAGGCGCATCATAGCAAT360
GluGlyCysAspAlaThrCysGlnPheArgLysAlaIleAspAspCysGlnLysGlnAlaHisHisSerAsn

GTTTTGCAGACTTCTGTACAAACAACTGCAACATTCACATCAATGGATACCTCCCAACTACCTGGAAATAGT432
ValLeuGlnThrSerValGlnThrThrAlaThrPheThrSerMetAspThrSerGlnLeuProGlyAsnSer

GTCTTCAAAGAATGTATGAAGCAGAAGAAAAAGGAATTTAGTTCAGGAAAGTAAAAGATTGAAGAAAATTGT504
ValPheLysGluCysMetLysGlnLysLysLysGluPheSerSerGlyLys---

AGCCGAGGAGAGAAAGAAAGAAAGTCCCATACCATATTTTGTTTGTTAATTGTAACGAATTTTCCGAAA
                                                    3'UT primer


Leader Peptide- MKQILLISLVVVLAVFAFNVAEG  23

Maxadilan- CDATCQFRKAIDDCQKQAHHSNVLQTSVQTTATFTSMDTSQLPGNSVFKECMKQKKKEFSSGK  63
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FIG. 2. **Nucleotide sequence and amino acid translation of the maxadilan gene.** *A*, nucleotide sequence and translation of the PCR-amplified cDNA fragment generated with the 3–13 primer (*overlined*) and the oligo(dT) primer (binding site *underlined*). A *Y* in the 3–13 primer sequence indicates both C and T at those positions. *B*, nucleotide sequence and translation of the PCR-amplified maxadilan gene fragment generated with an upstream primer (not shown) and the 3′-UT primer (binding site *underlined*). The positions of the A, B, C, and D primers are *overlined*. The putative TATAA box is *double underlined* and the putative cap site is marked with an *asterisk*. *Dots* indicate the position of the intron. *Underlined* amino acids identify residues 3–13, the region where maxadilan amino acid sequence was obtained. As the cDNA sequence in *A*, is a subset of the genomic sequence in *B*, nucleotide positions are indicated only for the latter and references in the text to specific positions, either cDNA or genomic sequence, refer to *B*. *C*, predicted amino acid sequence of mature maxadilan and its leader peptide in *single letter code*.

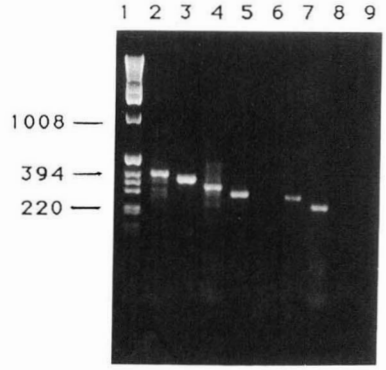

FIG. 3. **Intron and 5′ end of maxadilan mRNA predicted by PCR.** *Lane 1*, 1000-base pair ladder. *Lanes 2–5*, *L. longipalpis* genomic DNA amplified with upstream primers A–D and a downstream primer as described in the text and Fig. 2*B*. *Lanes 6–9*, *L. longipalpis* cDNA amplified with the same primers.

amplify genomic and cDNA (Fig. 3). As predicted, while genomic DNA was amplified with all four primers A, B, C, and D, cDNA was amplified successfully only with primers B and C. Sequencing of these amplified genomic and cDNAs confirmed the existence of the exon and intron shown in Fig. 2*B* and revealed the splice acceptor to be within the genomic DNA immediately upstream of the 3–13 primer-binding site. The inability of the A primer to produce a PCR product from cDNA suggests that the 5′ end of the mRNA lies between the A and B primers. As stated above, the region approximately 30 base pairs upstream of the putative cap site contains a canonical TATAA promoter sequence.

Based on the NH_2-terminal sequencing data and the cDNA sequence, maxadilan has a typical secretory leader of 23 amino acids (Fig. 2*C*) and there is no evidence of further amino-terminal processing. Mature maxadilan is predicted to contain 63 amino acids and have a molecular mass of 7026. This mass is 187 daltons greater than the mass predicted by mass spectrometry of the purified sand fly maxadilan. The reason for the slight discepancy in mass is not yet clear but may result from post-translational processing in the salivary gland. A potential phosphorylation site is present at the threonine at position 35. No asparagine-linked glycosylation sites are predicted. Computerized homology comparisons using GenBank release 67.0, PIR release 28.0, and SwissProt 18.0 have found no significant homology to other proteins.

Expression and Biological Activity of Recombinant Maxadilan—Expression of recombinant maxadilan was achieved by introducing the predicted coding DNA for mature maxadilan into the bacterial expression vector pGEX-3X (9) such that it was expressed in *E. coli* as a fusion protein with glutathione *S*-transferase. Following induction, the fusion protein was purified from bacterial extracts by glutathione-agarose affinity. The maxadilan peptide was then released from the fusion protein by activated factor X, which cleaves at its four amino acid recognition sequence present at the fusion junction, and the recombinant peptide purified to homogeneity on reverse-phase HPLC (Fig. 4). Between 2 and 10 mg of recombinant maxadilan has been obtained from 1-liter cultures.

The vasodilator activity of sand fly salivary gland extracts and purified natural maxadilan has been previously demonstrated by observing the development of erythema following superficial injection into rabbit skin (2). A similar assay on a human volunteer was performed with purified recombinant maxadilan peptide alongside a preparation of pure salivary gland maxadilan (Fig. 5). Injection of as little as 25 pg (approximately 4 fmol) of the recombinant peptide produced erythema within 30 min which persisted for hours. When 10 ng were injected into skin, equivalent to the amount of vasodilator in the extract of a pair of salivary glands, an area of erythema, psuedopods, and a blanched halo developed. There was no itching and almost no edema and the erythema from the injection of 10 ng persisted for more than 48 h. This pattern is identical to that which occurs following the bite of the sand fly (Fig. 5). Similar experiments have been performed several times in both humans and rabbits, and the limiting

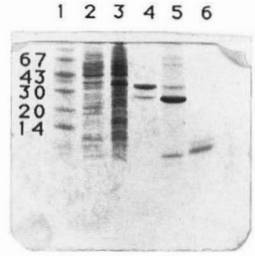

FIG. 4. **Expression of recombinant maxadilan.** *Lane 1*, molecular weight markers. *Lane 2*, extract from uninduced *E. coli*. *Lane 3*, extract from induced *E. coli*. *Lane 4*, single-step purification of fusion protein, M_r 33,000, with glutathione-agarose beads. *Lane 5*, Factor Xa cleavage of fusion protein yielding 26-kDa glutathione *S*-transferase and 7-kDa maxadilan. *Lane 6*, HPLC-purified recombinant maxadilan.

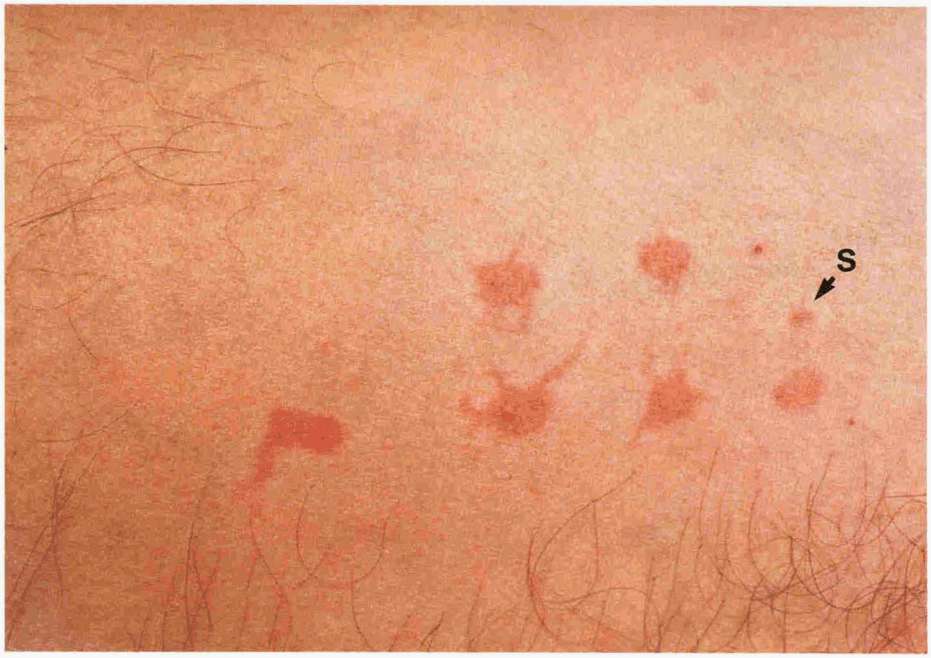

FIG. 5. **Erythema in human skin from a sand fly bite, HPLC-purified natural maxadilan, and the recombinant molecule.** The *left-most red spot* is from a sand fly bite. The next three areas of erythema represent the injection of 10 ng, 0.5 ng, and 25 pg of recombinant maxadilan. The *upper three areas* represent the injection of 10 ng, 0.5 ng, and 25 pg of natural maxadilan. The *S* indicates injection of phosphate-buffered saline. Note the rim of pallor and psuedopod formation, particularly from the sand fly bite and the higher doses of maxadilan.

dilutions for the recombinant maxadilan are consistently comparable to those obtained with the purified salivary maxadilan.

DISCUSSION

We report the isolation and characterization of genomic DNA and cDNA clones encoding maxadilan, the complete amino acid sequence of maxadilan and the functional recombinant expression of this potent vasodilator peptide from sand fly salivary glands. One approach to understanding the biological role and mechanism of action of this vasodilator is to compare the structure and physiological effects of maxadilan to other known vasoactive mediators. Vasoactive neuropeptides such as neurokinin A, B, vasoactive intestinal peptide, and substance P show no structural similarity with maxadilan and, unlike maxadilan, produce a wheal in addition to vasodilation upon injection (10). Bradykinin also has no apparent structural similarity to maxadilan and, unlike maxadilan, is associated with pain (11). Endothelin, a 21-residue molecule with two pairs of disulfide bonds, is the most potent vasoconstrictor peptide known (12). It does not share primary sequence homology with maxadilan but similarity on a tertiary level is possible. In this scenario, testable by competitive binding studies, maxadilan would act as an antagonist at the endothelin receptor (13, 14).

CGRP is the only known peptide which produces a pattern of irregular erythema and a halo without associated edema, itching, or pain, similar to maxadilan (2). CGRP is also a long-lasting vasodilator with a potency in the low picomole range (15). In addition, previous work has suggested that maxadilan has biochemical, immunochemical, and pharmacological similarities with CGRP (3). Based on these observations, CGRP has been studied to test whether other properties of sand fly saliva, in addition to erythema, might be mimicked by CGRP. These studies have shown that CGRP, like saliva, can inhibit the antigen presentation and γ-interferon activation of macrophages (6) and can dramatically enhance the infectivity of *Leishmania* (5). Taken together, these results suggest that maxadilan and CGRP bear a close functional relationship.

Because of this functional relationship, a careful comparison of the amino acid sequences of CGRP and maxadilan was performed to look for evidence of a structural relationship. Some apparent primary structural similarity can be found between the active CGRP peptide and both the NH_2-terminal and COOH-terminal portions of maxadilan as shown in Fig. 6. Secondary structure prediction using the method of Garnier *et al.* (16) also reveals distinct similarities (data not shown) but, in total, the similarities remain insufficient to conclude that a functional relationship of these two molecules results from structural similarity. In this regard, it will be important to test whether maxadilan can compete with CGRP for its receptor. Interestingly, both maxadilan and CGRP have highly charged COOH termini although this region is cleaved during the processing pathway of CGRP (Fig. 6). A synthetic maxadilan peptide lacking these eight COOH-terminal residues has a reduced specific activity, more like CGRP than full-length maxadilan, based on dose-response studies similar to those shown in Fig. 5. A testable hypothesis is that the charged COOH terminus is important to biological activity and that the COOH-terminal processing of CGRP actually reduces the potency of this molecule. Optimal alignment of the COOH terminus of maxadilan with CGRP also leaves two extra amino acids at the COOH terminus. It is worth noting that if these two amino acids were removed by a processing enzyme, the resulting maxadilan peptide has a molecular mass

```
                    N-terminal Alignment

HCGRP1     1 ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKA-F
               :: ::: ... .  .  .  . :.. :.: . : :
MAXADIL    1  CD-ATCQFRKAIDDCQKQA--HHSNVLQTSVQTTATFTSMDTSQLPGNSVFKECMKQKKKEFSSGK

                    C-terminal Alignment

HCGRP1     1 ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF///GRRRRDLQA
                 :::  .   . : . :  :.:. .        .....
MAXADIL   28 VQTTATFTSMDTSQLPGNS--VFKECM----------KQKKKEFSSGK
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FIG. 6. **Primary structure similarity between maxadilan and CGRP.** Weak homology can be found between both the NH_2-terminal and COOH-terminal halves of maxadilan (*MAXADIL*) and mature human CGRP1 (*HCGRP1*). Exact matches are indicated by a *colon* and similar amino acids are indicated by a *single dot*. The COOH-terminal alignment also shows the HCGRP1 sequence preceding the COOH-terminal processing site (///). Gaps introduced to maximize homology are indicated by a *dash*. The *numbers* preceding the amino acid sequences indicate the amino acid position.

of 6841, nearly identical to the mass predicted by mass spectrometry.

In summary, recently available biochemical techniques have allowed for the isolation, gene cloning, and expression of a novel vasodilator peptide from sand fly salivary glands. Maxadilan is a new tool with which to study vasodilation and this peptide, or mutants derived from it, could have pharmacological and vaccine applications. In addition, the techniques employed here could be used to study other pharmacologically potent molecules from arthropod salivary glands.

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