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The Blood-brain Barrier: Principles for Targeting Peptides and Drugs to the Central Nervous System

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Abstract

The presence of the blood-brain barrier (BBB), reduces the brain uptake of many drugs, peptides and other solutes from blood. Strategies for increasing the uptake of drugs and peptide-based drugs include; structural modifications to increase plasma half-life; improving passive penetration of the BBB by increasing the lipophilicity of the molecule; designing drugs which react with transporters present in the BBB; and reducing turnover and efflux from the central nervous system (CNS).

Introduction

The blood-brain barrier (BBB) is a vital element in the regulation of the constancy of the internal environment of the brain. The composition of the extracellular fluid of the brain is controlled within very precise limits, largely independently of the composition of the circulating blood, to provide a stable environment in which the integrative neuronal functions of the brain can optimally take place. The blood-brain barrier is formed at the level of the endothelial cells of the cerebral capillaries. These cells are characterised by having tight continuous circumferential junctions between the cells of the capillaries thus abolishing any aqueous paracellular pathways between the cells (Brightman 1992). The endothelium is thus characterised by exhibiting a high transendothelial electrical resistance in the region of 1500–2000 Ω cm^2 (Butt et al 1990). The presence of the tight junctions and the lack of aqueous pathways between cells greatly restricts the movement of polar solutes across the cerebral endothelium.

Some regions within the central nervous system (CNS) lack a BBB and the capillaries are fenestrated allowing the free movement of solutes between the blood and the surrounding interstitial fluid. These areas are collectively termed the circumventricular organs (CVOs) and comprise the choroid plexus, the median eminence, the neurohypophysis, the pineal gland, the organum vasculosum of the lamina terminalis, the subfornical organ, the subcommisural organ and the area postrema. Some of these structures, the median eminence, the neurohypophysis and the pineal are neurohaemal organs specialised for the release of neuroendocrine secretion into the bloodstream. The other areas may be regarded as windows of the brain where a limited number of neurones within the immediate vicinity of the circumventricular organ have an unrestricted access to blood solutes. This access enables the brain to monitor closely the composition of the blood and to react accordingly. The ependymal cells surrounding the circumventricular organs have what appear to be tight junctions between them presumably to enclose a volume of brain extracellular fluid (ECF) surrounding the CVO to prevent diffusion of interstitial solutes away from the region of the circumventricular organ (Brightman 1992). The relative surface area of the permeable fenestrated capillaries of the circumventricular organs compared to the tight BBB capillaries is 1 : 5000. Given these considerations there is little possibility of these high permeability areas being able to influence the composition of the bulk of the brain extracellular fluid and the CVOs do not form a realistic route for drug entry into the brain.

The presence of the BBB is easily demonstrated by studying the distribution of the inert hydrophilic marker inulin (Begley 1992a, 1994) (Table 1). The percentage distribution spaces in brain regions with a BBB correspond to the plasma volume of the brain area. In the choroid plexus and the anterior and posterior pituitary where the capillaries are fenestrated the inulin space corresponds to the extracellular space of the tissue.

Because of the presence of the BBB a number of specific transport mechanisms are required to be present in the cerebral endothelial cells to ensure that the brain receives an adequate supply of nutrients. These are illustrated and described in Fig. 1.

Passive diffusion may occur across the endothelium, either through the cells themselves or through the tight junctions between cells. Movement across the BBB by passive mechanisms will be determined by considerations such as molecular weight and lipophilicity as discussed later. The entry of glucose into the brain is via a facilitated carrier GLUT-1 present in the endothelial cells. GLUT-1 is insulin insensitive and always shows a relatively high level of expression. It will however upregulate in chronic hypoglycaemia and downregulate in chronic hyperglycaemia.

Amino acids are transported across the BBB on a variety of transporters. Large neutral amino acids, such as tyrosine, phenylalanine, leucine, isoleucine, valine, histidine and methionine are transported into the brain by an energy-dependent transporter termed system-L which is present on the luminal and abluminal membranes and is principally directed from blood to endothelial cell and from endothelial cell into brain. System-ASC which transports as model substrates alanine, serine and cystine, plus a number of neutral amino acids such as threonine and asparagine is also expressed to a lesser extent and with the same directional properties as system-L. There is an obvious

Table 1. Percent inulin spaces in various regions of the guinea-pig brain determined 10 min after intravenous bolus injection.

Brain region	Inulin space (%)
Whole brain	1·77 ± 0·14
Olfactory bulb	2·30 ± 0·37
Hippocampus	1·45 ± 0·22
Caudate nucleus	1·26 ± 0·18
Parietal cortex	1·50 ± 0·18
Hypothalamus	2·45 ± 0·30
Choroid plexus	20·80 ± 1·10
Pituitary (anterior & posterior)	26·70 ± 2·90

The differences between brain area possessing a blood-brain barrier and areas where the capillaries are fenestrated may be illustrated by injecting experimental animals with radiolabelled inulin (5000 Da). Where there is a blood-brain barrier the inulin occupies the plasma space of the tissue which is between 2.45 and 1.26% depending on the region. Where the capillaries are fenestrated the inulin occupies the plasma and the extracellular space of the tissue giving spaces of approximately 25%. The inulin spaces were determined after an intravenous bolus injection of 50mCi [^{3}H]inulin in anaesthetised guinea-pigs. After 10 min, blood samples were taken by cardiac puncture and the animals were decapitated. Brain samples were then dissected out and the inulin space calculated as: inulin space = $C_{plasma}/C_{brain} \times 100$. Mean ± s.e.m.

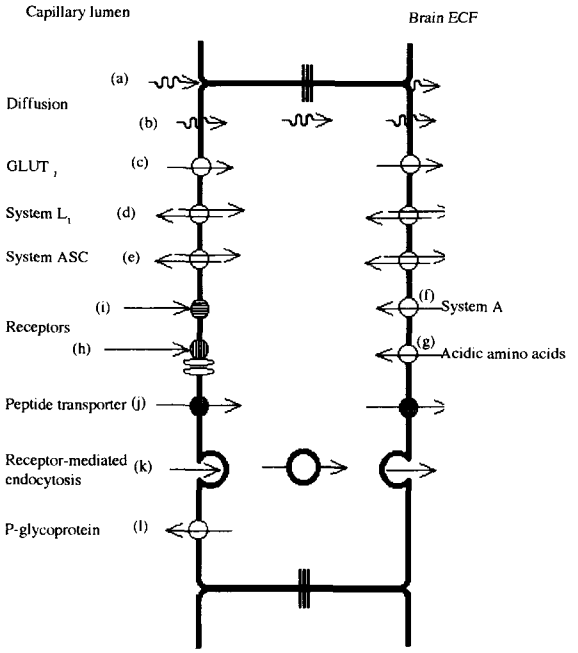


FIG. 1. Interactions of solutes with the blood-brain barrier. Solutes both in blood and in the brain extracellular fluid may interact with the BBB in a variety of ways. Solute which traverse the BBB by diffusion may either diffuse to a limited extent through the zona occludens (a) or directly across the endothelium (b). Polar solutes, for example glucose, employ a facilitated carrier Glut-1 (c), in the cell membrane and neutral amino acids employ Systems-L and ASC (d and e). Glut-1, system-L and System ASC are represented in the same orientation in both the luminal and the abluminal membranes of the cerebral capillary endothelial cells. System-A (f) transports glycine and a system for the acidic amino acids (g) transports glutamic acid and aspartic acid from the brain extracellular fluid into the cerebral endothelial cells. There are also receptors for blood-borne solutes on the luminal surface of the endothelium (i and h). These receptors may be linked to intracellular messengers or control the activity of other channels in the luminal membrane and thus alter the activity of the endothelial cells. Specific peptide transporters have been demonstrated in the luminal endothelial membrane (j) transporting peptides into the endothelium. If these peptides are to reach the brain extracellular fluid by this route there must be equivalent transporters in the abluminal membrane. Larger peptides and proteins will be internalized by an endocytic mechanism (k). This may be receptor-mediated or non-specific in nature. The level of endocytic activity in the cerebral capillary endothelium is much lower than in other tissues endothelia and transcytosis remains a controversial topic. Multidrug resistance protein or P-glycoprotein is expressed constitutively in the luminal membrane of the cerebral endothelial cells (l) and transports a variety of structurally unrelated substrates out of the endothelium. Many of these are lipophilic and potentially neurotoxic and would otherwise enter the brain to a greater extent.

overlap in the substrates acceptable to system-L and ASC. System-A, transporting principally glycine and proline is present on the abluminal membrane of the endothelial cells and is directed out of the brain (Betz & Goldstein 1978). A transporter for the dicarboxylic amino acids, glutamic and aspartic acid, is also present in the BBB and is directed out of the brain. Both system-A and system-ASC are sodium- as well as energy-dependent. A number of specific receptors for solutes also exists both on the luminal and abluminal surfaces of the endothelial cells. These receptors may be linked to second messengers such as cAMP or may modulate the activity of channels or transporters in the BBB. There may also be transporters in the cell membrane for solutes such as small peptides.

The level of endocytic activity in the BBB, compared with other endothelia, is minimal. However transcytosis for certain macromolecules may occur and form a low capacity transport mechanism for these solutes. Receptor-mediated endocytosis may be solute-specific and inducible by that solute.

The BBB also has a physiological and biochemical dimension in that the endothelial cells have a high density of mitochondria compared with other endothelia presumably reflecting a high level of oxidative ATP production. In addition the BBB is the site of a high level of enzyme activity directed towards the inactivation of centrally active blood-borne solutes and toxins (Audus et al 1992; Grieg 1992). The high enzyme activities present in or on the cerebral endothelial cells include monoamine oxidase (MAO) types A and B, L-amino acid decarboxylase (AAD), catechol-*O*-methyl transferase (COMT), butyryl-cholinesterase (BChE) and 4-aminobutyrate aminotransferase. Levels of gamma-glutamyl transpeptidase activity are high in cerebral endothelial cells and are thought to be related to amino acid transport phenomena. Also membrane-bound epoxidehydrolase (mEH), UDP-glucuronosyl-transferase (UGT), benzoxyresorufin-*O*-de-ethylase, NADPH cytochrome P450 reductase and glutathione-*S*-transferase activities are high in brain microvessel preparations and in the choroid plexus. These enzymes are thought to play a part in the metabolism of drugs and xenobiotic compounds.

The intrinsic membrane protein P-glycoprotein sometimes referred to as multidrug resistance protein (mdr-protein) is also present in the luminal plasma membrane of the endothelial cells constituting blood-brain barrier. This is an efflux pump which appears to be a constituent part of the BBB and transports a wide range of structurally unrelated substances out of cells. Pgp is an ATP-dependent pump and is a member of a family of intrinsic membrane proteins which are normally expressed at the BBB, the intestine, the liver and the kidney (Cordon-Cardo et al

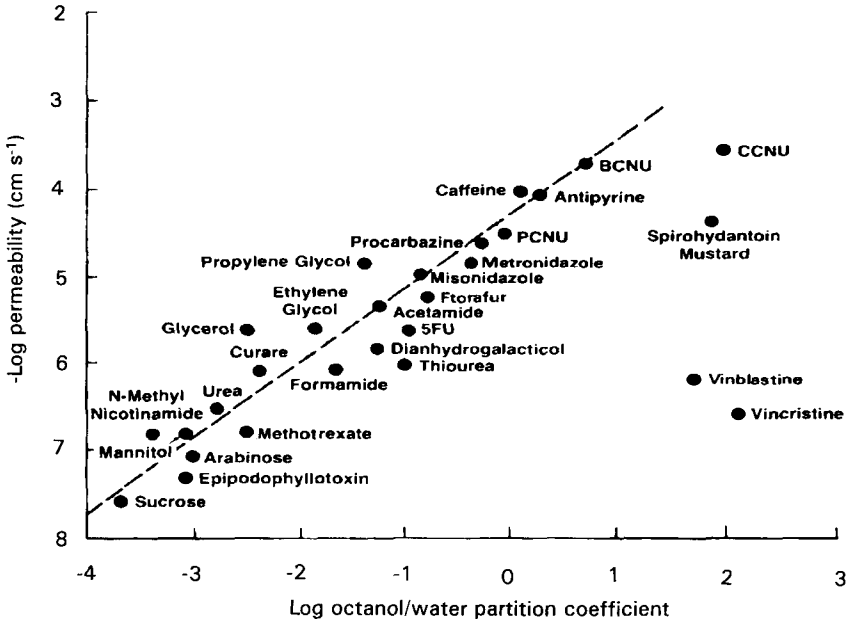


FIG. 2. The relationship of the octanol/water partition coefficient to blood-brain barrier permeability for some selected drugs. Substances falling on the dotted line would have a direct relationship between their brain uptake and their lipid solubility. Note that there is a wide scatter of points around the line. Vinblastine and vincristine are known substrates for the efflux pump P-glycoprotein expressed at the blood brain barrier and this limits their penetration into brain. Other substances which also lie well away from the line but are not shown on the plot are those which have carrier-mediated uptake mechanisms such as glucose and amino acids, which are fairly polar with partition coefficients between 1×10^{-2} and 1×10^{-4} but with high BBB permeabilities.

BCNU = 1.3-bis-chloro(2-chloroethyl)-1-nitrosourea; CCNU = 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; PCNU = 1-(2-chloroethyl)3-(2,6,-dioxo-3-piperidyl)-nitrosourea; 5FU = 5-fluorouracil. Adapted from Greig (1992).

1989; Ruez & Gros 1994). The role of Pgp therefore appears to be a protective one transporting a range of potentially toxic substances out of the body. The expression of Pgp occurs in many tissues including malignant tissue after exposure to cytotoxic drugs where it confers resistance to a wide range of cytotoxic agents. Hence its alternative name multidrug resistance protein. In this situation its acts to maintain the intracellular levels of cytotoxic drugs below a toxic level and thus frustrates repeated cancer chemotherapy. Its constitutive role in the normal BBB is inferred to be a protective one reducing the entry of lipophilic and neurotoxic substances into the CNS.

Improving passive permeation of the BBB

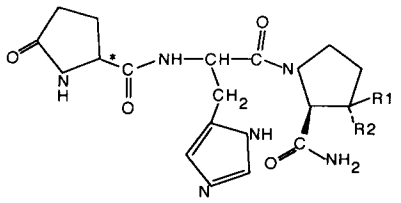
For many drugs that enter the brain by passive diffusion their brain-uptake and lipid solubility are well correlated (Fig. 2). Lipid solubility is most often determined as a partition coefficient between buffer and octanol and is quoted as the logarithm of the partition, $\log P_{oct}$. Many small solutes entering the brain do so by dissolving in the lipid of the cell membrane. In-vitro studies using cultured cerebral endothelial cells have suggested that in this system the relationship between lipid solubility and brain uptake might be a sigmoidal one (Oldendorf 1974; Van Bree et al 1988). With substances having a logP between −2.00 and 0 (partition coefficients of 0·01 and 1·00) lying on the more linear portion of the curve. Very hydrophobic substances show little further increase in brain uptake and hydrophilic substances which are polar and highly ionized at physiological pH also have a limited brain uptake.

It has been suggested by Levin (1980) that large lipid-soluble substances with a molecular weight above 500 Da become physically impeded from crossing the cell membrane and thus exhibit a molecular weight cut-off. This has been used to explain the low brain-uptake of substances such as vinblastine, vincristine and cyclosporin, which lie well off the regression line suggested in Fig. 2. However it is now well-established that these cytotoxic agents are substrates of P-glycoprotein (Tsuji et al 1992, 1993) which is actively extruding them from the cerebral endothelial cells. Surprisingly an increase in molecular volume may actually enhance brain uptake (Abraham et al 1994).

It has been suggested that delta logP, defined as the $\log P_{octanol/buffer}$ minus the $\log P_{cyclohexane/buffer}$ is a better predictor of brain uptake (Young et al 1988). Delta logP reflects the hydrogen-bonding capacity of a substance and this property in turn influences brain uptake. In general in a series of related substances those with a lower-hydrogen bonding potential enter the brain the most readily. The hydrogen-bonding potential determines the activation energy necessary to pluck a molecule out of the aqueous phase and into the lipid phase of a membrane. Various physical factors influencing brain uptake have been incorporated into a predictive equation for brain uptake (Abraham et al 1994).

To maximize the brain uptake and the bioavailability of a substance to the brain a number of molecular manipulations may be carried out to alter the properties of a substance; these may be summarized as:

1. Increasing the plasma stability and hence plasma half life.
2. Improving lipid solubility.



(a) pyroglutamyl-histidyl-prolinamide (TRH)
R1 = H, R2 =H

(b) pyroglutamyl-histidyl-3 methylprolinamide (Pyr-His-Mep-NH_2, RX 74355)
R1 = H, R2 = CH_3

(c) pyroglutamyl-histidyl-3,3 dimethylprolinamide (Pyr-His-Dmp-NH_2, RX 77368)
R1 = CH_3, R2 = CH_3

FIG. 3. Structure of TRH and related analogues RX 74355 and RX 77368. The substitution of the methyl groups into the proline residue increases the plasma half-lives and the central effectiveness of the molecules.

3. Enhancing or maintaining reactivity with existing BBB transport mechanisms.
4. Retaining central nervous activity.
5. Increasing the stability in brain extracellular fluid and reducing reactivity with efflux transport mechanisms in the CNS.

A number of chemical modifications can be carried out to improve the CNS activity of drugs. For example the peptide thyrotropin releasing hormone (TRH—pyroglutamyl-histidyl-prolinamide) has a short plasma half life due to circulating peptidases. To extend the half-life, methyl groups may be added to the prolinamide residue to produce a 3-methylprolinamide (RX74355) and a 3,3-dimethylprolinamide (RX77368) (Brewster et al 1981, 1983) (Fig. 3). These methyl substitutions increase the lipid solubility of the molecule and also the molecular volume. The substitutions also increase the first order half-lives in human plasma from 33 (TRH) to 210 (RX74355) and 1080 min (RX77368) (Table 2). The central nervous activity of the TRH analogues is also enhanced (Brewster et al 1981; Brewster 1983), possibly as the result of increased brain penetration although an enhanced central potency should also be considered.

Other simple modifications to peptides that may be made are to amidate the C-terminus and acetylate the N-terminus both of which confer an increased resistance to exopeptidases. In addition acetylation of the N-terminus also increases lipid solubility significantly.

An excellent example of increasing brain uptake by enhancing lipid solubility and reducing hydrogen bonding capacity is the chemical conversion of morphine to heroin (diacetyl-morphine). Substitution of the two hydroxyl groups of morphine by acetyl groups in heroin increases the brain uptake over twenty-fivefold (Oldendorf 1974). As a general rule for each pair of hydrogen bonds removed from a molecule there is a log order increase in BBB permeabilty. Once within the brain, heroin is rapidly converted to monoacetyl morphine and more slowly to morphine. Thus the rapid brain entry of heroin in comparison with morphine makes it a favoured drug of abuse and presumably enhances its addictive potential.

In the case of many peptides the introduction of D-isomers of the naturally occurring amino acids in to the peptide sequence can greatly enhance the plasma half life of the peptide. Again the reactivity with plasma peptidases is greatly reduced. For example octreotide (D-Phe-Cys-Phe-D-Tyr-Lys-Thr-Cys-Thr-OH : sandostatin : SMS 201-995) is an octapeptide analogue of somatostatin Ala-Gly Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH). The attenuated peptide with the D substitutions, D-Phe at position two of somatostatin and D-Trp at position five, extends the first order half-life of somatostatin from 2-3 min in human plasma to 113 min (Lamberts 1987). The substitution of D-Phe at position four also increases the growth hormone inhibiting potency of sandostatin compared with somatostatin by some 45 times. D-Amino acid substitutions also have marked effects on the plasma half-lives of encephalin analogues. Examples are two analogues of leucine encephalin (Tyr-Gly-Gly-Phe-Leu), namely DADLE

Table 2. First-order half-lives (min) of TRH, RX74355, and RX77368. There are significant species differences in the rates at which plasma and a brain homogenate will break down TRH and its analogues.

	TRH	RX74355 (Methylproline)	RX77368 (Dimethylproline)
Rat			
Plasma	22	114	390
Brain homogenate	9	54	190
Dog			
Plasma	>1500	>1500	>1500
Brain homogenate	11	90	174
Man			
Plasma	33	210	1080
Brain homogenate	18	66	168
Mouse			
Plasma	215	>1500	>1500
Brain homogenate	12	28	150

From Brewster (1983).

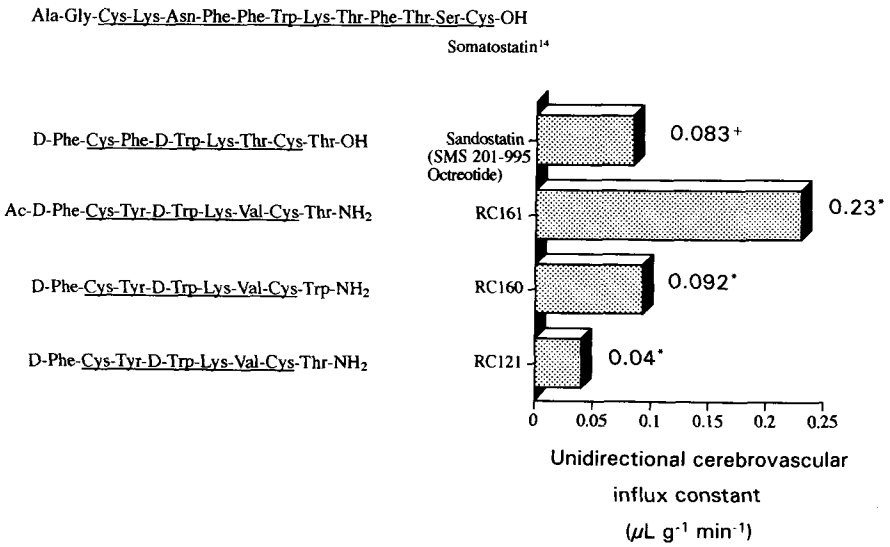


FIG. 4. Unidirectional cerebrovascular permeability constants (K_{in}) of some somatostatin analogues. The brain uptakes are determined by intravenous bolus injection techniques. The structure of the analogues is given with that of somatostatin for comparison. Unfortunately the brain uptake of somatostatin cannot be determined by a comparable method as it is very unstable in plasma. Data from Begley et al (1992b) and Banks et al (1990).

(D-Ala^2, D-Leu^5-encephalin) and dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg) both of which have greatly extended half-lives in plasma compared with the parent peptide.

A number of somatostatin analogues exist where the plasma half-life is extended and the lipid solubility is altered thus modifying two factors which influence brain uptake, plasma half life and biological potency of the molecules. Intravenous bolus injection studies have been carried out to measure the brain uptake of these peptides, (Banks et al 1990; Begley 1992b, 1994). These somatostatin analogues are amphiphilic and their brain uptakes are non-saturable suggesting that their penetration into the brain will be passive. However changes in the molecular structure of the analogues produce significant changes in brain uptake. Fig. 4 illustrates the brain uptakes of four octapeptide analogues of somatostatin, determined by an intravenous bolus injection technique (Banks et al 1990; Begley 1992b, 1994) which have D-Phe and D-Trp substitutions in the molecule. Compared with RC 121 the substitution of a Trp residue at position 8 compared with RC 160, thus introducing an additional aromatic side chain into the molecule, approximately doubles the cerebrovascular permeability constant. Acetylating the N-terminus of RC 121 as in RC 161, and therefore increasing the lipid solubility, increases the permeability constant almost sixfold. The peptide RC 160 has marked and prolonged analgesic actions after intravenous administration (Eschalier et al 1990).

Using synthetic peptide sequences which have hydrophobic properties may provide a mechanism for inserting a peptide into the cell membrane (Begley 1994). For example a number of naturally-occurring peptides have hydrophobic regions which demonstrate a natural affinity for cell membranes. Mellitin, a component of bee venom (Kaiser & Kezdy 1987), is a 26-amino acid peptide (Fig. 5). The N-terminal amino acids 1–20 contain two α-helical regions 1–10 and 13–20 which are hydrophobic and insert the molecule into the cell membrane, the lysine residues 21–26 are highly cationic and are thought to open up pores thus permeabilizing the membrane (Kaiser & Kezdy 1987).

Signal peptides are similar hydrophobic regions in a pre-pro-peptide or protein which enable the entire molecule to be inserted through the unit membrane of the endoplasmic reticulum. The signal amino acid sequence is not conserved between pre-pro-proteins and thus the common feature is the hydrophobicity (lipohilicity) of the region (Engelman & Steitz 1981; Emr & Silhavey 1983; Begley 1994). The

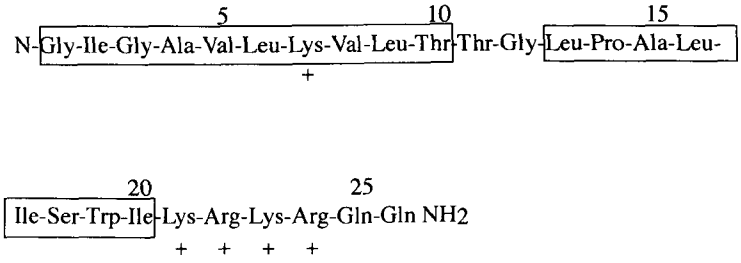


FIG. 5. Amino acid sequence of mellitin. The hydrophobic areas which insert into the cell membrane are boxed. Positively-charged side chains which disrupt the cell membrane are indicated with +.

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