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Polymyxin B Is an Inhibitor of Insulin-induced Hypoglycemia in the Whole Animal Model

STUDIES ON THE MODE OF INHIBITORY ACTION*

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The cyclic decapeptide, polymyxin B (PMXB), was found to inhibit hypoglycemia in mice receiving exogenous insulin (Amir, S., and Shechter, Y. (1985) *Eur. J. Pharmacol.* 110, 283–285). In this study, we have extended this observation to rats. Insulin-dependent hypoglycemia in rats is efficiently blocked at a 12:1 molar ratio of PMXB to insulin. This effect is highly specific, as it could not be mimicked by a variety of antibiotics or positively charged substances. Chemical modifications of PMXB have revealed that the ring structure, rather than the tail structure, is important for anti-insulin-like activity. Colistin A, which differs from PMXB by one conservative amino acid substitution in the ring structure, is devoid of this activity. Polymyxin B does not interact with insulin, nor does it alter the rate of insulin absorption and/or degradation, or the ability of insulin to bind to target tissues. This peptide inhibits hypoglycemia by blocking insulin-dependent activation of the hexose transport mechanism, as deduced by *in vitro* studies. The effect of insulin in stimulating hexose uptake (and subsequent glucose metabolism) in both isolated muscle tissue and adipocytes is blocked with little or no effect on the basal activities of these processes. Colistin A has no significant inhibiting effect. Other insulin-dependent activities, such as inhibition of lipolysis in adipocytes or synthesis of DNA in muscle cells, are not inhibited. It is concluded that PMXB inhibits, in a highly specific manner, the action of insulin in stimulating hexose transport and subsequent glucose metabolism, both *in vitro* and in the whole animal model.

In the last decade, impressive achievements have been made with respect to the structure, basic properties, and behavior of the insulin receptor (reviewed Refs. 1 to 3). In spite of this, the mechanism(s) of insulin action, in particular the postbinding events involved in hormone action, are largely unknown. This in part is due to the lack of an insulin-responsive cell-free system, as most or all the actions of the hormone are lost upon cell disintegration (reviewed in Ref. 4). Therefore, agents or conditions that either mimic or inhibit the effects of insulin may be of use in elucidating the mechanism(s) of insulin action. Several classes of membrane perturbants (reviewed in Ref. 5) are known to mimic the action of insulin by directly perturbing the receptor or interfering with events proximal to the insulin receptor sites. These agents may have a limited value in elucidating the postbinding mechanism(s) involved in hormone action. In contrast, vanadate ions, which mimic insulin by specifically modulating certain cytoplasmic events (6–9), may be of more use. Conditions or agents that inhibit the actions of insulin are rare. Some examples include those conditions under which cellular ATP concentrations are depleted (10–12). In addition, various anti-calmodulin drugs are known to directly inhibit insulin action (13, 14). Inhibition of insulin action by calmodulin inhibitors suggests that either calmodulin or a related Ca^{2+}-dependent regulatory protein may participate in one of the events leading to glucose entry and/or metabolism. These inhibitors, however, do not interfere with the ability of insulin to antagonize lipolysis in the same cells (13).

In a preliminary communication (15), we have demonstrated that polymyxin B sulfate (PMXB),[1] a cyclic decapeptide antibiotic, inhibits the effect of exogenously administered insulin in mice. The anti-insulin action of PMXB is long lasting (>12 h), prevents hypoglycemia and mortality in mice challenged with a lethal dose of insulin, and is not mimicked by many substances which resemble PMXB in character and structure (15). Even colistin A sulfate (polymyxin E), which differs from PMXB by a single conservative amino acid substitution (see Fig. 1 for structure of PMXB and colistin), is ineffective in inhibiting insulin-mediated hypoglycemia (15).

To the best of our knowledge, this is the first demonstration of a peptide that can block the action of insulin in the whole animal. In the present study, we extend our preliminary observation, seen in mice, to rats. We have also elucidated the mechanism of the inhibitory action of PMXB. Since colistin A resembles PMXB in structure, charge, and hydrophobic character, but lacks its inhibitory capacity, colistin was used as a control substance in many of the experiments described below.

EXPERIMENTAL PROCEDURES

Materials—Bovine and porcine insulin were purchased from Sigma. D-[U-^{14}C]Glucose (4–7 mCi/mol), 2-D-[G-^{3}H]deoxyglucose (9 Ci/

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§ Incumbent of the Henry Glasberg Career Development Chair.

§§ Incumbent of the C. H. Hollenberg Chair in Metabolic and Diabetes Research established by the Friends and Associates of Dr. C. H. Hollenberg of Toronto, Canada. To whom correspondence and reprint requests should be addressed.

[1] The abbreviations used are: PMXB, polymyxin B; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DAB, diaminobutyric acid; MOA, 6-methyloctanoic acid; Ac-His$_2$-PMXB, a PMXB derivative in which 2 residues of *N*-acetylhistidine were covalently attached to the amino groups of the PMXB moiety; KRB buffer, Krebs-Ringer bicarbonate buffer.

mmol), and 2-D-[1,2-^{3}H]deoxyglucose (30.2 Ci/mmol) were from New England Nuclear. [U-^{14}C]Sucrose (552 mCi/mmol) and [^{125}I]iodine (carrier-free) were obtained from Amersham. Polymyxin B sulfate was purchased from Sigma and colistin A from Rafa Israel. Collagenase type I (134 units/mg) was obtained from Worthington. All other materials used in this study were of analytic grade.

Derivatization of PMXB—Succinyl$_5$-PMXB and acetyl$_5$-PMXB were prepared by applying a 200 molar excess of either succinic anhydride or acetic anhydride, respectively, to PMXB (20 mg in 1.0 ml of 1 M $NaHCO_3$). Anhydride addition was performed in aliquots of 5 mg each, over a period of 1 h at 5 °C. Acetyl$_2$-PMXB and succinyl$_2$-PMXB were prepared by applying a 10 molar excess of either acetic anhydride or succinic anhydride under the above-mentioned conditions. All derivatives were purified on a Sephadex G-10 column (36 cm × 1.5 cm) pre-equilibrated with 0.1 M NaCl. Derivatives were eluted from the column with the same 0.1 M NaCl solution. The number of amino groups modified by anhydride treatment was determined by the trinitrobenzenesulfonic acid method (16). The derivative, Ac-His$_2$-PMXB, was prepared by coupling PMXB to *N*-acetylhistidine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. The reaction mixture (1.0 ml) contained 20 mM PMXB, 800 mM *N*-acetylhistidine, and 40 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. The reaction was allowed to occur for 5 h at pH 7.0–7.5. The derivative was then purified on a Sephadex G-10 column, using 0.1 M NaCl as the elution buffer. An aliquot of the eluate was then acid-hydrolyzed, and its amino acid composition was determined. Under these conditions, it was found that 2 mol of acetylhistidine were incorporated per mol of PMXB. Iodination of insulin or Ac-His$_2$-PMXB using [^{125}I]iodine was performed using the chloramine-T method (17).

Measurement of 2-D-Deoxyglucose Uptake—Measurement of 2-deoxyglucose uptake in isolated rat soleus muscles was done using male albino rats of the Hebrew University strain (approximately 40 g/rat). Animals were killed by cervical dislocation, and the soleus muscles were quickly removed and placed under an O_2:CO_2 (95:5 v/v) atmosphere in 10-ml glass vials sealed with rubber stoppers. The muscles were then incubated in a metabolic shaker (40 cycles/min) at 37 °C. Groups of four muscles each were incubated for 5 min in KRB buffer, supplemented with 10 mM HEPES and 10 mM D-glucose in the presence or absence of either PMXB (100 μg/ml) or colistin (100 μg/ml). The muscles were further incubated for 15 min at 37 °C in the presence of porcine insulin (0.5 μg/ml). Isolated muscles were then washed with 20 ml of KRB buffer, transferred to vials containing KRB with bovine serum albumin (0.25% w/v), 0.5 mM 2-deoxyglucose, 30 μCi of [^{3}H]2-deoxyglucose, and 1 μCi of [^{14}C]sucrose, and were incubated for an additional 5 min. Hexose uptake was then terminated by three rapid washes with 20 ml of ice-cold KRB buffer containing 5 μM cytochalasin B.

Treated muscles were then frozen in liquid nitrogen and weighed. After thawing, individual muscles were dissolved in 1 ml of 1 N NaOH at 70–80 °C for 10 min. Heated samples were then cooled and neutralized with concentrated HCl and were counted for radioactive content. Net intracellular 2-deoxyglucose uptake was calculated as the difference between the distribution of [^{3}H]2-deoxyglucose and the extracellular marker, [U-^{14}C]sucrose.

Additional Procedures—The following methods and procedures were used with no modification: preparation of isolated fat cells from epididymal fat pads (18), lipogenesis (19), 2-D-deoxyglucose uptake in fat cells (20), lipolysis and its inhibition by insulin (21), binding of insulin to rat adipocytes (22), and thymidine incorporation into smooth muscle cells in culture (23). For plasma glucose determinations, mice were killed by decapitation and the trunk blood was collected and centrifuged to obtain plasma. Plasma glucose was determined using a Beckman Glucose Analyzer.

RESULTS

Antihypoglycemic Effects of PMXB in Rats—Similar to its action in mice (15), PMXB (see Fig. 1 for structural configuration) was found to inhibit the hypoglycemic actions of exogenously administered insulin in rats (Fig. 2). Complete inhibition of insulin action was obtained at a 12:1 molar ratio of PMXB to insulin. This inhibitory effect by PMXB was found to be highly specific, as none of the many other antibiotic or positively charged substances tested showed anti-insulin activity (not shown). Colistin A, which differs from PMXB by one conservative substitution in the ring structure (see Fig. 1 for structural configuration), was also found to not possess anti-insulin activity (Table I). Of the five amino groups of PMXB, only two could be modified without a significant loss of activity (Table I). Octapeptin (Des-DAB$_1$-Des-Thr$_2$-PMXB) and a PMXB-derivative in which the ultimate tail MOA and the penultimate DAB were removed, Des-MOA-Des-DAB$_1$-PMXB, had 100% and 40% of the original antihypoglycemic activity of PMXB (Table I), respectively. The latter derivative is devoid of the antibacterial action of PMXB (24).

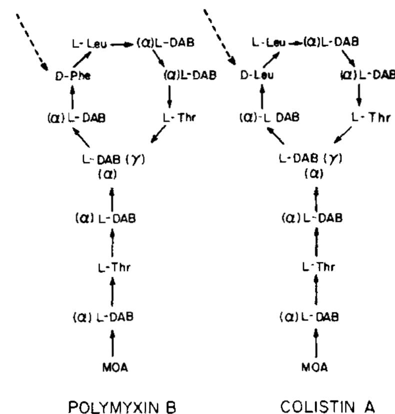

Fig. 1. The peptide structure of polymyxin B and colistin A. The *arrows* indicate the sole amino acid moiety which differentiates PMXB from colistin A.

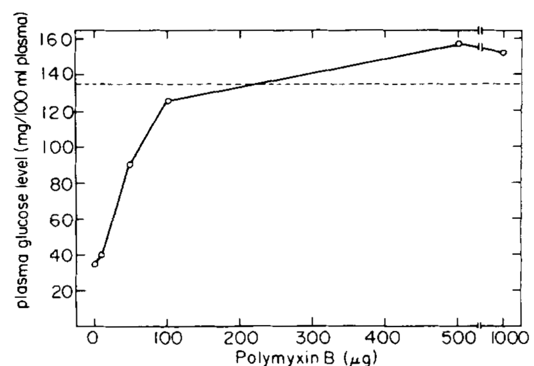

Fig. 2. The effect of polymyxin B on insulin-induced hypoglycemia in rats. Male Wistar rats (100 g) were injected subcutaneously with either 100 μg of insulin or with insulin plus increasing doses of PMXB, as indicated. The rats were killed 60 min later, and the plasma glucose levels were then determined. The *dashed line* indicates the mean plasma glucose levels of control rats. Each group consisted of six rats.

Cellular Mechanism(s) of Polymyxin B Inhibition of Hypoglycemia—In an attempt to understand the mechanism(s) of the inhibitory action of PMXB on insulin-induced hypoglycemia in both mice and rats, we tested for the following possible conditions. Firstly, we examined the possibility of a direct interaction occurring between PMXB and insulin which might lead to inactivation of the hormone. Secondly, we examined for any possible impairment of insulin absorption into the blood circulation, and, finally, we looked for a direct action of PMXB on insulin responsiveness in target tissues.

Preincubation of insulin with a 100-fold PMXB did not result in any significant reduction in the ability of insulin to stimulate lipogenesis (Table II). Also, PMXB does not appear to form complexes with insulin, as judged by equilibrium dialysis measurements (Table II), or by the "peak and trough"

TABLE I

Relative activity of polymyxin B and several polymyxin B derivatives in inhibiting insulin-induced hyperglycemia

Both insulin (2.5 μg/mouse) and/or increasing concentrations of PMXB or its derivatives were administered by subcutaneous injections. Plasma glucose levels were then determined 15 min after treatment.

Substance	Relative activity[a]
	%
PMXB	100
Colistin A	0–7
Ac-His_2-polymyxin B[b]	93–100
$Succinyl_2$-polymyxin B[c]	95–100
$Acetyl_2$-polymyxin B[d]	94–100
$Succinyl_5$-polymyxin B[e]	0–7
$Acetyl_5$-polymyxin B[f]	0–5
Des-MOA-Des-DAB_1-polymyxin B[g]	37–43
Des-DAB_1-Des-Thr_2-polymyxin B[h] (octapeptin)	95–105

[a] The concentration of PMXB which leads to half-maximal inhibition of insulin-mediated decreased plasma glucose levels was taken as 100%.

[b] A PMXB derivative containing an average of 2 mol of DAB-linked *N*-acetylhistidine per mol of PMXB.

[c] A polymyxin B derivative containing 2 mol of succinyl moieties per mol of PMXB.

[d] A polymyxin B derivative containing 2 mol of acetyl moieties per mol of PMXB.

[e] A polymyxin B derivative in which all five amino groups were succinylated.

[f] A polymyxin B derivative in which all five amino groups were acetylated.

[g] A polymyxin B derivative in which the terminal MOA and DAB_1 were removed.

[h] A polymyxin B derivative which does not contain either DAB_1 or Thr_2.

method (not shown). Moreover, PMXB does not appear to interfere with insulin binding either to liver membranes or to rat adipocytes (Table II). It seems then that PMXB does not complex with or inactivate insulin in any way.

We have found that the rate of insulin absorption into the blood circulation is the same in the presence of either PMXB or its inactive analog, colistin (Fig. 3). Co-administration of either PMXB or colistin with insulin does not appear to enhance either absorption or cleavage of the hormone. Within 30 min of treatment, about 15–20% of the hormone remains intact, as judged by trichloroacetic acid precipitability and radioimmunoassay measurements (Table III).

Next we tested the effects of PMXB on insulin responsiveness in rat adipocytes. PMXB treatment does not interfere with isoproterenol-dependent lipolysis in rat adipocytes, nor with insulin inhibition thereof (Table IV). On the other hand, PMXB, but not colistin A, abolished the action of insulin in stimulating 2-deoxyglucose uptake (Table IV). Basal levels of glucose uptake were only slightly affected (not shown). Supporting these results, we further demonstrated that PMXB also inhibits insulin-stimulated lipogenesis (Fig. 4). Basal rates of lipogenesis were also affected by PMXB treatment, but to a much lesser extent. Colistin (100 μg/ml) appears to have a slight inhibitory effect on insulin-stimulated lipogenesis (about 20% of that of PMXB), but had a negligible effect on basal levels of lipogenesis (Fig. 4). Half-maximal inhibition of lipogenesis was obtained at a PMXB concentration of 30 μg/ml in a representative experiment (Fig. 4).

Inhibition of Insulin-mediated 2-Deoxyglucose Uptake in Rat Soleus Muscle—As muscle tissue has a major role in absorbing circulating glucose in response to an insulin challenge in the whole animal, it was essential to determine whether PMXB blocks this process in this target tissue. The

TABLE II

Lack of interaction of polymyxin B with insulin and with insulin receptor

	ED_{50} value of insulin
	ng/ml
Insulin inactivation	
Native insulin	0.15 ± 0.03
Insulin pretreated with 100-fold excess of PMXB[a]	0.15 ± 0.02
	Equilibrium dialysis[b]
	cpm/0.1 ml
Formation of insulin-PMXB complexes	
Radioactivity inside tube	44,000 ± 500
Radioactivity outside tube	44,800 ± 700
	Specific binding of ^{125}I-insulin
Interference with ^{125}I-insulin binding	
Rat adipocytes	23 ± 2 fmol/10^6 cells
Rat adipocytes + PMXB (0.8 mg/ml)	22 ± 2 fmol/10^6 cells
Liver membranes	3.0 ± 0.4 fmol/20 μg protein
Liver membranes + PMXB (0.8 mg/ml)	3.1 ± 0.3 fmol/20 μg protein

[a] Insulin (2 μg) and PMXB (200 μg) were incubated in 60 μl of 0.1 M phosphate buffer for 5 h. The mixture was then diluted to yield various concentrations of insulin, ranging between 0.01 and 10 ng/ml, and was tested for its ability to stimulate lipogenesis. Insulin at 10 ng/ml gave a 5-fold stimulation over basal activity. ED_{50} values were derived from the dose-response curves obtained.

[b] Insulin (1.0 ml of 0.6 mg/ml insulin in 0.1 M $NaHCO_3$ (pH 7.8)) was dialyzed for 12 h at room temperature against a 1-ml solution containing 2 × 10^{-4} M PMXB and [^{125}I]iodo-His_2-PMXB (880,000 cpm) in the same buffer. Aliquots (in triplicate) were then taken for determining their radioactive content.

[c] Binding was performed for 1 h at 22 °C using 1 nM ^{125}I-insulin (for fat cells) or 3 nM ^{125}I-insulin (for liver membranes) in the presence or the absence of 5 μM unlabeled insulin. Results are expressed as femtomoles of [^{125}I]iodoinsulin bound per aliquot of protein or cell number.

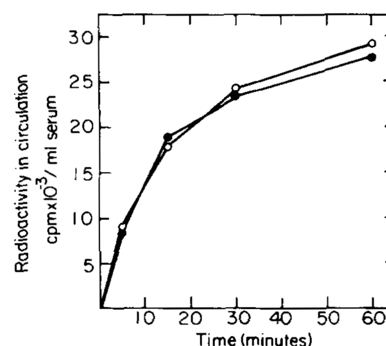

FIG. 3. **Effects of PMXB and colistin on the circulatory absorption of radiolabeled insulin.** Bovine insulin (2.5 μg/mouse), together with [^{125}I]iodoinsulin (1.3 × 10^6 cpm/mouse) were subcutaneously injected together with either PMXB (250 μg/mouse, ○) or colistin (250 μg/mouse, ●). At the indicated times, groups of mice were killed, and the amount of radioactivity in the plasma was determined.

results, summarized in Table V, indicate that PMXB, at 100 μg/ml, completely blocks the effect of insulin in stimulating 2-deoxyglucose uptake. Basal levels of glucose uptake were not affected by PMXB. In contrast, colistin, at 100 μg/ml, did not have any effect on either basal or insulin-stimulated glucose uptake.

TABLE III

Processing of exogenously administered insulin in the rat after its co-administration together with colistin A or PMXB

Mice were injected each with 5 μg of bovine insulin containing 2 × 10^6 cpm of [^{125}I]iodoinsulin (250,000 cpm/ng), together with 500 μg of PMXB or 500 μg of colistin. Mice were killed after 30 min. The trunk blood was collected and centrifuged to obtain plasma.

	Total insulin: intact + degraded[b] (after 30 min)	Trichloroacetic acid-precipitated protein (after 30 min)	Intact insulin[a] (after 30 min)	Radioimmunoassay[c]
	ng/ml	*%*	*ng/ml*	
Insulin + colistin	247	15	37.05	22
Insulin + PMXB	228	16	36.5	19.5

[a] Determined by trichloroacetic acid precipitability.
[b] Determined by radioactive content of the serum.
[c] Determined by standard double-antibody radioimmunoassay.

TABLE IV

Effect of PMXB and colistin on insulin-mediated actions in rat adipocytes

	Glycerol released	Inhibition
	nmol/3 × 10^5 cells/h	*%*
Inhibition of lipolysis		
None	10 ± 1	
PMXB (100 μg/ml)	10 ± 0.8	
Isoproterenol (0.02 μM)	125 ± 7	
Isoproterenol + insulin (3.0 nM)	30 ± 2	83
Isoproterenol + insulin + PMXB	33 ± 2	80
	Uptake of 2-deoxyglucose	
	nmol/10 min/10^6 cells	
Glucose uptake[a]		
None	0.71 ± 0.04	
Insulin (17 nM)	1.84 ± 0.1	
Insulin + colistin (100 μg/ml)	1.72 ± 0.12	
Insulin + PMXB (100 μg/ml)	0.73 ± 0.07	

[a] A suspension of adipocytes was incubated with the indicated concentrations of colistin or PMXB for 35 min at 37 °C, and with insulin for 30 min at the same temperature. 2-D-Deoxyglucose uptake was then measured for 10 min at 37 °C.

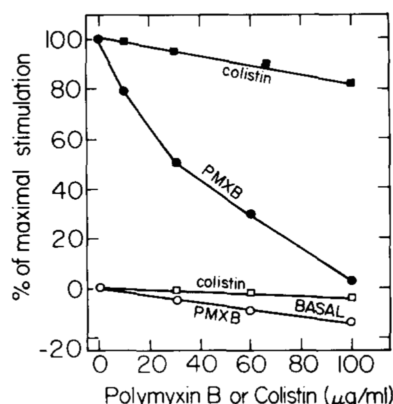

FIG. 4. **Effects of increasing concentrations of polymyxin B and colistin on the active and the basal state of lipogenesis.** Lipogenesis was carried out in the absence (○, □) or the presence (●, ■) of insulin (100 ng/ml) and increasing concentrations of colistin or PMXB.

TABLE V

Inhibition of 2-deoxyglucose uptake in rat soleus muscle by polymyxin B

Experimental conditions were as described.

Additions	2-Deoxyglucose incorporated
	fmol/mg/5 min
None	19.10 ± 2.26 (4)
Polymyxin B (100 μg/ml)	22.5 ± 1.28 (4)
Insulin (0.5 μg/ml)	32.46 ± 2.16 (4)
Insulin (0.5 μg/ml) + PMXB (100 μg/ml)	20.02 ± 1.52 (4)
Colistin (100 μg/ml)	19.00 ± 0.41 (4)
Insulin (0.5 μg/ml) + colistin (100 μg/ml)	34.73 ± 2.53 (3)

TABLE VI

Effect of polymyxin B and insulin on DNA synthesis in cultured smooth muscle cells

Addition[a]	[^{3}H]Thymidine incorporated[b]	-Fold stimulation
	cpm/7.7 × 10^4 cells	*%*
None	9,200 ± 250	100
Insulin (17 nM)	33,600 ± 2,500	365 ± 27
PMXB (10 μg/ml)	15,800 ± 530	172 ± 6
Insulin (17 nM) + PMXB (10 μg/ml)	52,800 ± 2,000	574 ± 22

[a] Insulin or PMXB were incubated with cells for a total of 24 h to which labeled [^{3}H]thymidine was added for the last 16 h of incubation.
[b] Cells were subcultured and grown in 35-mm plates containing 3 ml of Dulbecco's modified Eagle's medium-0.2% fetal bovine serum for 3 days before exposure to either insulin or PMXB.

Actions of PMXB on Insulin-mediated Synthesis of DNA in Smooth Muscle Cells—Polymyxin B at 10 μg/ml does not inhibit the action(s) of insulin in mediating long term biological effects, such as DNA synthesis (shown in a representative experiment, Table VI). In fact, PMXB itself may have some promoting effect upon DNA synthesis (about 27% of that obtained by insulin). This stimulating effect by PMXB appears to be additive to the effect of insulin.

DISCUSSION

Our studies show that PMXB is able to counteract insulin-induced hypoglycemia in both mice and rats. From our experiments, we conclude that the ring structure of the peptide contains the essential domain(s) involved in anti-insulin action. While the mechanism of the inhibitory action is not completely understood, our studies were aimed at testing several possible modes of action. The observation of the lack of interaction between PMXB and insulin (Table II) implies that the observed inhibitory effect is not a result of the inability of the hormone to reach its target tissue and bind to specific receptor sites. The metabolic degradation of insulin, when administered with PMXB or the inactive analog, colistin (Table III), was identical in either case and suggests that the inhibitory peptide does not modify the rate of absorption or endogenous processing of the hormone. The possibility that PMXB triggers a mechanism which results in an increase in blood glucose levels was also excluded, since PMXB alone did not affect blood glucose levels at any point in time following its administration (15). Moreover, PMXB was fully active in adrenalectomized mice and in mice pretreated with 6-hydroxydopamine,[2] guanethidine,[3] phentolamine,[4] propranolol,[5] or

[2] The catecholamine neurotoxin, 6-hydroxydopamine, was applied to mice, intravenously at 50 mg/kg, 48 h before testing.
[3] Guanethidine, the catecholamine depletor, was applied intravenously at 10 mg/kg, 24 h before testing.
[4] Phentolamine, the α-adrenergic blocker, was applied intraperitoneally at 10 mg/kg, 30 min before testing.
[5] Propranolol, the β-adrenergic blocker, was applied intraperitoneally at 10 mg/kg, 30 min before testing.

indomethacin[6] (not shown). Thus, it is unlikely that PMXB modulation of secondary endogenous substances, such as adrenal hormones, sympathetic amines, and prostaglandins, is part of the inhibitory process. PMXB, however, was found in *in vitro* studies to specifically block insulin-dependent glucose uptake and its metabolism in insulin-responsive tissues, such as fat or muscle. Other activities of insulin, *i.e.* the inhibition of lipolysis in fat cells or DNA synthesis in smooth muscle cells, were also unaffected by the peptide.

Taking into consideration our *in vivo* and *in vitro* observations in both mice and rats, we propose that PMXB exerts its inhibitory action at a post-receptor site which is specific to the activation of glucose uptake, and not to other insulin-dependent activities. To our knowledge, PMXB is the only compound capable of specifically blocking insulin-induced glucose uptake in both *in vitro* and *in vivo* systems, and, as such, may prove to be a useful experimental tool for studying the mechanism of action of insulin in normal and pathological states.

The post-receptor site of PMXB action is currently under investigation in our laboratory. Kunn *et al.* (25–27) have previously demonstrated that PMXB binds to acidic phospholipids of various mammalian tissues. Initial attempts are being undertaken to identify an insulin-dependent post-binding event that may be perturbed by PMXB-phospholipid interactions.

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REFERENCES

1. Jacobs, S., and Cuatrecasas, P. (1981) *Endocr. Rev.* **2,** 251–263
2. Shechter, Y. (1985) in *The Receptors* (Conn, M. P., ed) Vol. 2, pp. 221–224, Academic Press, Orlando, FL
3. Czech, M. P. (1985) *Annu. Rev. Physiol.* **47,** 357–381
4. Czech, M. P. (1977) *Annu. Rev. Biochem.* **46,** 357–381
5. Czech, M. P. (1980) *Diabetes* **29,** 399–409
6. Shechter, Y., and Karlish, S. J. D. (1980) *Nature* **284,** 556–558
7. Degani, H., Gochin, M., Karlish, S. J. D., and Shechter, Y. (1981) *Biochemistry* **20,** 5795–5799
8. Dubyak, G. R., and Kleinzeller, A. (1980) *J. Biol. Chem.* **255,** 5306–5312
9. Tamura, S., Brown, T. A., Whipple, J. H., Yamaguchi, Y.-F., Dubler, R. E., Cheng, K., and Larner, J. (1984) *J. Biol. Chem.* **259,** 6650–6658
10. Chandramouli, U., Milligan, M., and Carter, J. R., Jr. (1977) *Biochemistry* **16,** 1151–1158
11. Kono, T., Robinson, F. W., Sarver, J. A., Vega, F. V., and Pointer, R. H. (1977) *J. Biol. Chem.* **252,** 2226–2233
12. Siegel, J., and Olefsky, J. M. (1980) *Biochemistry* **19,** 2183–2190
13. Shechter, Y. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 327–331
14. Begum, N., Tepperman, H. M., and Tepperman, J. (1986) *Endocrinology* **118,** 287–292
15. Amir, S., and Shechter, Y. (1985) *Eur. J. Pharmacol.* **110,** 283–285
16. Habeeb, A. F. S. A. (1966) *Anal. Biochem.* **14,** 328–336
17. Hunter, W. M., and Greenwood, F. C. (1962) *Nature* **194,** 495–496
18. Rodbell, M. (1964) *J. Biol. Chem.* **239,** 375–380
19. Moody, A. J., Stan, M. A., Stan, M., and Gliemann, J. (1974) *Horm. Metab. Res.* **6,** 12–16
20. Olefsky, J. M. (1976) *J. Clin. Invest.* **57,** 842–851
21. Shechter, Y. (1982) *Endocrinology* **110,** 1579–1583
22. Gliemann, J., Osterland, K., Venter, J., and Gammeltoft, S. (1972) *Biochim Biophys. Acta* **286,** 1–9
23. Kaiser, N., Tur-Sinai, A., Hasin, M., and Cherasi, E. (1985) *Am. J. Physiol.* **249,** E292–E298
24. Vaara, M., and Vaara, T. (1983) *Antimicrob. Agents Chemother.* **24,** 107–113
25. Kunin, C. M. (1970) *J. Infect. Dis.* **121,** 55–64
26. Kunin, C. M., and Bugg, A. (1971) *J. Infect. Dis.* **124,** 394–400
27. Craig, W. A., and Kunin, C. M. (1973) *J. Pharmacol. Exp. Ther.* **184,** 757–765

[6] Indomethacin, the inhibitor of prostaglandin synthesis, was applied intraperitoneally at 50 mg/kg, 60 min before testing.