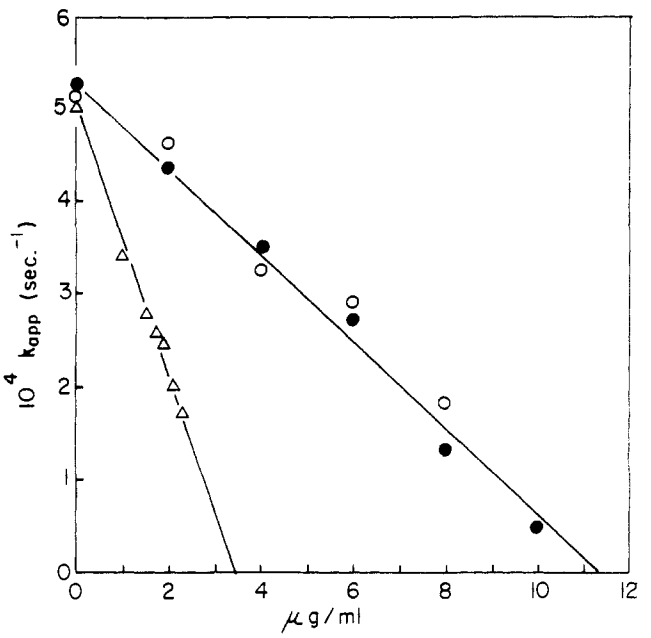

Figure 1. Plots of apparent microbial generation rate constants as a function of antibiotic concentration: chloramphenicol (△); *N*-trifluoroacetyl analogue of D-*threo*-chloramphenicol, old lot (○), new lot (●).

uated by microbial kinetics in the same manner[1] at pH 7 and compared to chloramphenicol. The results are given in Figure 1 and it can be seen that the inhibitory rate constant of **1**, $k = 4.63 \times 10^{-5}$ mL/μg/s, was 0.32 the value for **2**; $k = 1.45 \times 10^{-4}$ mL/μg/s. This value for **1** was tenfold less than that previously reported (see Figure 2 in ref 1) whereas the value for **2** was similar to that reported previously.

The data in the original laboratory notebooks were then carefully reviewed and a probable transcription error was observed. It was noted on one page that two stock solutions of **1** were prepared at 20.7 mg/100 mL and 51 mg/25 mL (0.207 and 2.04 mg/mL, respectively) and it was stated that dilutions of the former were used in the microbial kinetic studies where the results were tabulated on a different page for compound **1**. It was apparent that the latter solution was actually used and it can be properly presumed that the concentrations used in the calculations were based on the premise that they were one-tenth the actual concentrations. Thus the inhibitory rate constant for **1** was erroneously reported as being tenfold its actual value.

Careful review of the original data in the laboratory notebook indicates that there is little probability of any other errors in the reported microbial kinetic data given in the published paper[1] for the 36 other chloramphenicols studied and it can be concluded that none of the analogues had greater potencies than chloramphenicol.

References and Notes

(1) C. Hansch, K. Nakomato, M. Gorin, P. Denisevich, E. R. Garrett, S. M. Heman-Ackah, and C. M. Won, *J. Med. Chem.*, 16, 917 (1973).
(2) Personal communication, K. Freeman, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada.
(3) C. Hansch, Pomona College, Claremont, Calif. 91711.

Evidence for Separate Peptide Sequences Related to the Lipolytic and Magnesium-Accumulating Activities of ACTH. Analogy with Adrenergic Receptors

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Native adrenocorticotropin [ACTH-(1–39)] and ACTH-(1–24) stimulate both lipolysis and magnesium accumulation in rat adipocyte plasma membrane vesicles. ACTH-(1–20) retains full lipolytic activity but has a minimal effect on magnesium accumulation. In contrast ACTH-(11–24) stimulates magnesium accumulation but not lipolysis. These findings indicate that within the ACTH molecule the peptide sequence responsible for stimulation of magnesium accumulation is distinctly separate from the core sequence (residues 4–10) essential for stimulation of adenylyl cyclase activity and cAMP mediated lipolysis. Phentolamine, an α-adrenergic antagonist, blocks the bulk of magnesium accumulation stimulated by native ACTH and norepinephrine; propranolol, a β-adrenergic antagonist, blocks the earliest phase of Mg^{2+} uptake by these hormones but has little effect on net uptake. Isoproterenol, a β-adrenergic agonist, stimulates magnesium uptake only minimally. The pattern of uptake stimulated by methoxamine, an α-adrenergic agonist, or ACTH-(11–24) is quite similar to that produced by native ACTH in the presence of propranolol. The receptor through which ACTH mediates stimulation of the bulk of magnesium appears to be analogous to the α-adrenergic receptor through which norepinephrine stimulates this same process.

Epinephrine, norepinephrine, and ACTH have recently been reported to stimulate ATP-dependent magnesium accumulation in plasma membrane vesicles prepared from rat adipocytes.[1] Previous studies of ACTH have correlated its structure with steroidogenesis, binding to receptors, antigenicity, and lipolysis. Phylogenetic studies of ACTH have revealed an invariant region (residues 1–24) and a variable region (residues 25–39), which is more antigenic. The invariant region contains the core sequence (residues 4–10) identified with stimulation of adenylyl cyclase.[2] ACTH-(15–18) contains a sequence of basic residues important for binding to the target cell.[3,4] Residues 19–24 have not been investigated except insofar as they protect ACTH-(1–24) from degradation.[5]

In this study we compare the effects on Mg^{2+} accumulation of ACTH-(1–20), ACTH-(1–24), and ACTH-(11–14) with the parent peptide ACTH-(1–39). The importance of residues 21–24 for optimal stimulation of magnesium uptake has been indicated previously.[5] We are now able to show that the core sequence, necessary for the stimulation of adenylyl cyclase, is not required.

Some insight into the nature of the receptor involved in magnesium accumulation has been gained through the use of adrenergic antagonists.[5] These studies utilizing agonists as well as antagonists indicate that an α-adrenergic mechanism is involved not only in the action of nor-

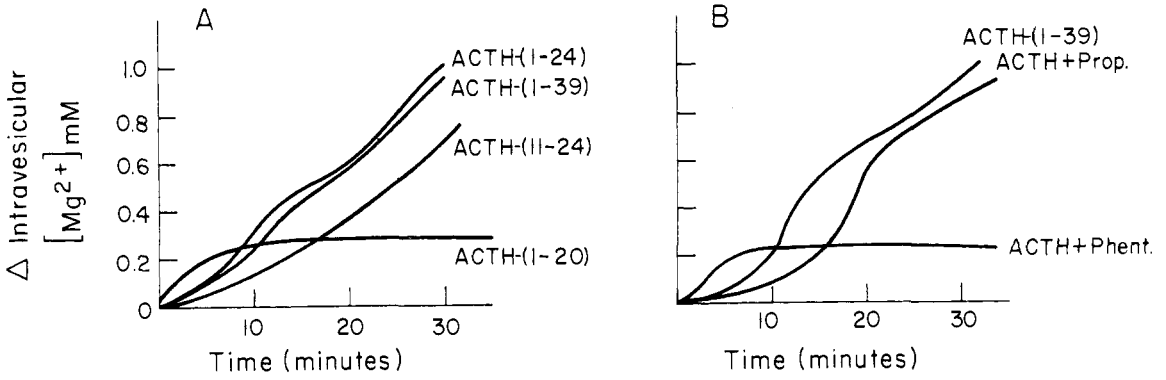

Figure 1. Accumulation of magnesium by adipocyte plasma membranes as a function of time after the addition of various ACTH peptides and adrenergic antagonists. This continuous recording of 8-hydroxyquinoline-5-sulfonic acid fluorescence was calibrated to indicate magnesium accumulation.

Table I. Effect of ACTH Peptides on Magnesium Accumulation in Adipocyte Plasma Membranes[a]

	Net increase in intravesicular $[Mg^{2+}]$/30 min, mM	Amt of Mg^{2+}/g of protein/30 min, μmol
ACTH-(1-39) [4]	0.94 ± 0.09	9.78 ± 0.94
ACTH-(1-24) [4]	0.95 ± 0.10	9.99 ± 1.04
ACTH-(11-24) [3]	0.80 ± 0.12	8.32 ± 1.25
ACTH-(1-20) [4]	0.36 ± 0.23	3.74 ± 2.39

[a] Conditions are described in the text. The numbers in brackets after each hormone are the number of observations in each group. The concentration of all hormones was 5×10^{-8} M. The activities of the first three peptides did not differ significantly from each other. The activity of ACTH-(1-20) was significantly less than that of the other peptides ($p < 0.05$).

epinephrine but also of ACTH as well and could account for the bulk of magnesium accumulation without stimulation of adenylyl cyclase.

Results

The results in Table I indicate that the residues 25–39 at the carboxy terminus and residues 1–10 at the amino terminus can be removed without a significant loss of the effect of ACTH on magnesium accumulation. Removal of residues 21–24, however, as in ACTH-(1–20), results in a reduction in this activity by almost two-thirds.

The effects of adrenergic agents on magnesium accumulation (Table II) indicate that α blockade (phentolamine) causes a significant reduction ($p < 0.05$) in magnesium accumulation of about 75%, while β blockade (propranolol) reduces accumulation by about 30% ($p < 0.05$). The activity of the α-agonist methoxamine does not differ significantly from that of norepinephrine, which is both an α- and β-agonist, or from norepinephrine plus propranolol. The β-agonist, isoproterenol, however, has less than 25% of the activity of norepinephrine ($p < 0.05$).

The time course of magnesium accumulation shown in Figure 1 (A) indicates that the small accumulation following ACTH-(1–20) occurs rapidly. The initial accumulation following ACTH-(11–24) is slower, but overall accumulation approaches that of ACTH-(1–24), which does not differ significantly from that caused by the natural peptide, ACTH-(1–39).

In the presence of phentolamine, stimulation of magnesium accumulation by the natural ACTH peptide is low and follows a pattern similar to that of ACTH-(1–20) [Figure 1 (B)]. Blockade by propranolol slows the initial phase of accumulation but has a minimal effect on overall accumulation [Figure 1 (B)].

Table II. Effect of Adrenergic Agonists and Antagonists on Magnesium Uptake in Adipocyte Plasma Membrane Vesicles[a]

Hormones	Net increase in intravesicular $[Mg^{2+}]$/30 min, mM	Amt of Mg^{2+}/g of protein/30 min, μmol
Norepinephrine[b]	1.30 ± 0.20	13.52 ± 2.08
Norepinephrine + propranolol[b]	0.85 ± 0.15	8.84 ± 1.56
Norepinephrine + phentolamine[b]	0.37 ± 0.20	3.85 ± 2.08
Methoxamine[b]	1.07 ± 0.19	11.13 ± 1.95
Isoproterenol[b]	0.30 ± 0.12	3.12 ± 1.25

[a] Conditions are described in the text. Concentrations used: norepinephrine 1×10^{-5} M; methoxamine 2×10^{-4} M; isoproterenol 1×10^{-5} M; propranolol 1×10^{-4} M; and phentolamine 2×10^{-4} M. [b] $n = 4$.

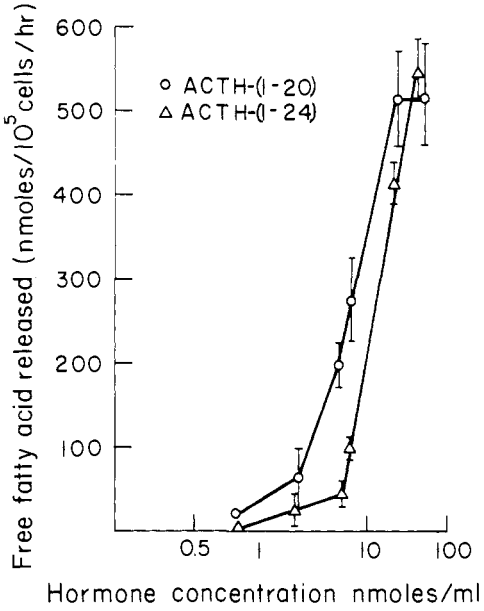

Figure 2. The effect of ACTH peptides on fatty acid release from isolated adipocytes. The assay procedure is described in the text.

Lipolytic activity is significantly greater for ACTH-(1–20) than for ACTH-(1–24) (Figure 2). ACTH-(11–24) has no significant lipolytic activity.

Discussion

ACTH-(11–24) lacks the core sequence (residues 4–10) responsible for the activation of adenylyl cyclase and has no significant lipolytic activity or steroidogenic activity; the peptide acts as a competitive inhibitor for ACTH-

(1–39) and -(1–10) in steroidogenesis.[10] ACTH-(11–24), however, retains most of the effect of native ACTH on magnesium accumulation. It appears, therefore, that in ACTH different sequences of residues are responsible for adenylyl cyclase activation and the major portion of magnesium accumulation. Residues 21–24 are probably the ones which play the most important role in magnesium accumulation, since ACTH-(1–20) stimulates this activity only minimally, while ACTH-(1–24) is as potent as the parent hormone.

The mechanism by which ACTH stimulates magnesium accumulation appears to be analogous to the action of α-adrenergic agents. The bulk of the effect of both ACTH and adrenergic agents on magnesium accumulation is blocked by α-adrenergic antagonists. The effect of ACTH-(11–24), which lacks a stimulatory effect on adenylyl cyclase, is similar to that of the α-adrenergic agonist, methoxamine, which also does not stimulate this enzymatic activity. Both substances fail to cause the rapid initial accumulation seen with norepinephrine, native ACTH, and isoproterenol. This accumulation is small in net amount and inhibited by β blockade. While the bulk of magnesium accumulation seen after hormone stimulation of adipocyte plasma membranes appears to operate through an α-adrenergic receptor, cAMP may mediate a much smaller accumulation of magnesium seen immediately after hormone stimulation.

Experimental Section

Materials. ACTH-(1–30) was obtained from Calbiochem, La Jolla, Calif. ACTH-(1–24) (Synacthen) and phentolamine (Regitine) was obtained from Ciba-Geigy Corp., Summit, N.J. ACTH-(1–20) was synthesized in our laboratories by the solid-phase method as previously described.[6] ACTH-(11–24) was generously supplied by Dr. W. Rittel, Ciba Geigy Corp., Basel, Switzerland. Norepinephrine was obtained from Sigma Chemical Co. L-Propranolol was obtained from Ayerst Laboratories, New York, N.Y., and methoxamine hydrochloride from the Wellcome Research Laboratories, Research Triangle Park, N.C.

Bioassays. Rat adipocytes were isolated as described by Cushman[7] using chromatographically purified collagenase obtained from Worthington Biochemical Corp., Freehold, N.J. Plasma membrane vesicles containing the fluorescent ligand 8-hydroxyquinoline-5-sulfonic acid were prepared from rat adipocytes and Mg^{2+} accumulation determined from the increase in fluorescence of the ligand as previously described.[1] ^{63}Ni was used to measure the release of fatty acid from isolated adipocytes by the method of Ho[8] as modified by Draper et al.[9]

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References and Notes

(1) D. A. Elliott and M. A. Rizack, *J. Biol. Chem.*, **249**, 3985 (1974).
(2) O. Hechter and T. Braun in "Structure–Activity Relationships of Protein and Polypeptide Hormones", M. Margoulies and F. C. Greenwood, Ed., Excerpta Medica, Amsterdam, 1972, p 212.
(3) J. Ramachandran and V. Lee, *Biochem. Biophys. Res. Commun.*, **41**, 358 (1970).
(4) K. Hofmann, J. A. Motibeler, and F. N. Finn, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 80 (1974).
(5) D. A. Elliott, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **33**, 1455 (1974).
(6) M. W. Draper, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **33**, 1455 (1974).
(7) S. W. Cushman, *J. Cell Biol.*, **46**, 326 (1970).
(8) R. J. Ho, *Anal. Biochem.*, **36**, 105 (1970).
(9) M. W. Draper, R. B. Merrifield, and M. A. Rizack, *J. Med. Chem.*, **16**, 1326 (1973).
(10) S. Seelig, G. Sayers, R. Schwyzer, and P. Schiller, *FEBS Lett.*, **19**, 232 (1971).

Quantitative Structure–Activity Relationships of Chymotrypsin. On the Predictive Value of Correlation Equations

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It is shown that inhibition constants against chymotrypsin for new congeners of the type $R_1C(=O)R_2$ are well predicted by a correlation equation published earlier.

Now that the field of quantitative structure–activity relationships (QSAR) has reached a modest level of maturity, it becomes of increasing interest to begin to more rigorously assess the predictive value of correlation equations. Toward this end we have recently surveyed the literature and summarized examples where previously formulated equations were later found to give good correlations with new data.[2] Rouot et al.[3] have recently illustrated the forecasting ability of QSAR with clonidine analogues. In addition, we have now shown that a QSAR for 102 antimalarials[4] gives good results for over 100 new analogues.[5] In this report we wish to show that a QSAR formulated[6] for the inhibition of chymotrypsin by inhibitors of the type $R_1C(=O)R_2$ applies to new data.

$$\log 1/C = 0.35\Sigma MR - 0.0099(\Sigma MR)^2 + 0.74(I\text{-}1) + 0.83(I\text{-}2) - 0.36(I\text{-}3) - 0.77(I\text{-}4) + 0.66 \quad (1)$$
$$n = 103;\ r = 0.944;\ s = 0.290$$

Equation 1 was formulated from data obtained by Baker and his students. In this equation, C is the molar concentration of inhibitor producing 50% inhibition and ΣMR refers to the sum of MR for R_1 and R_2 (scaled by 0.1). The compounds are ketones, amides, or esters. R_2 represents Me of methyl ketones or NHR or OR of amides or esters. In the large majority of cases, $R_1 = -CH_2OC_6H_4\text{-}X$. The