potassium phenethicillin in distilled water, so that each 5 μl. contains 5 units of phenethicillin activity.

Paper Strips and Equipment.—Prepare paper strips of suitable size from Whatman No. 1 chromatography paper, and mark them for application of the samples approximately 1 in. from the bottom. Use a 5-μl. pipet for application of samples to the spots. Any closed container, adapted for ascending paper chromatography, may be used as a developing tank. We use a 2-L. large-mouth, round, amber bottle with a screw cap. The strips are held in place by strings and clips inserted through holes in the cap and secured to the cap with adhesive tape. Pressurized Chromatosprayers, purchased from Research Specialties Co., Richmond, Calif., are used to apply the color reagent after development of the strips.

Procedure.—Apply the entire 0.1 ml. of test sample to one spot on the origin line of the strip, and the 0.1 ml. of control sample to an adjacent spot. Do not let the spot diameters exceed 0.8 cm. To a third spot, apply 5 μl. of phenethicillin standard (5 units). Allow the spots to dry, and then expose the strip to ammonia fumes in a closed container for 10 min. Remove and air dry. Place in the developing tank so that about 1/4 in. of the bottom of the strip is immersed in the mobile phase. Allow to develop to the 15-cm. mark, remove, and air dry. Again, place the strip in ammonia vapor for 10 min., remove, air dry, and spray *once lightly* with 0.02 *M* iodine solution. White spots on an iodine-colored background indicate the presence of phenethicillin, R_f approximately 0.55. The size and intensity of the spot produced by the control sample should be similar to those of the spot produced by the phenethicillin standard. Any sign of phenethicillin in the test sample indicates contamination, the degree of which is estimated by comparison of the three spots.

Discussion.—Treatment of the paper strip with ammonia fumes serves to split the lactam ring of the phenethicillin molecule, producing the thiazolidine derivative. The latter reacts with iodine, whereas the intact molecule does not (3). This step is carried out prior to development of the paper strip, because the thiazolidine derivative has a lower and more desirable R_f value than does phenethicillin itself. The authors have found that unwanted residues are carried along closer to the solvent front, and they tend somewhat to reduce the R_f value of phenethicillin and to distort the shape of the spot.

Some formulations provide excessive amounts of residues that thwart detection of phenethicillin. In some such cases, it has proved advantageous to add another extraction step, as follows.

Reduce the chloroform extract to 20 ml., and extract with two 10-ml. portions of 1% phosphate buffer, pH 6.0, to remove the phenethicillin. Combine the extracts, adjust them to pH 2.5 with 1 *N* hydrochloric acid, add 2 ml. of 10% phosphate buffer, pH 2.5, and extract with two 40-ml. portions of chloroform. Wash the chloroform extracts with 20 ml. of 1% phosphate buffer, pH 2.5, take to dryness with air and a warm water jacket, and proceed with the methanol extraction steps given under *Preparation of Test Sample.*

This method has been used successfully for a variety of pharmaceutical formulations, both simple and complex. It is considered a tentative procedure, because the authors realize that it will need modification in certain instances. It provides a quick inexpensive screening method and a starting point for further refinement.

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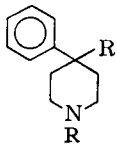
Linear Free Energy Relationship Among Analgesic *N*-Substituted Phenylpiperidine Derivatives. Method of Detecting Similar Modes of Molecular Binding to Common Receptors

Sir:

If compounds in two different series of analgesics are exerting their effect by interacting in a similar way with a common analgesic receptor, then identical changes in a portion of the molecule belonging to both series should produce parallel variation in activity. Thus, identical changes in the *N*-substituent in two series of analgesics

TABLE I.—REGRESSION ANALYSIS OF THE LOGARITHM OF THE ANALGESIC ACTIVITY IN VARIOUS *N*-SUBSTITUTED PHENYLPIPERIDINE SERIES



I, R′ = CO_2Et

II, R′ = OCO—Et

III, R′ = OCO—Me

Correlation[a]	Slope[b]	S.E.	S.D.	*r*[c]	*n*[d]	Data Source[e]
I *vs.* II[h]	1.20	0.09	0.16	0.99	5	*f*
	1.01	0.13	0.25	0.96	6	*g*
I *vs.* III[i]	0.93	0.24	0.37	0.90	4	*f*
	0.87	0.21	0.42	0.87	6	*g*
III *vs.* II[j]	1.09	0.20	0.27	0.96	4	*f*
	1.06	0.19	0.25	0.94	5	*g*

[a] Series I, II, and III were plotted as the logarithm of the activity (μM/Kg.). [b] Values were calculated by the method of least squares. [c] Represents the linear correlation coefficient; when $r = 1$, there is a perfect correlation; if $r = 0$, there is no correlation. [d] Denotes the number of points in the regression. [e] All data were obtained from *Reference 3*; mice were the test animals. [f] Eddy's data. [g] Janssen's data. [h] R = $\phi CH{=}CHCH_2$; $\phi(CH_2)_3$; $\phi(CH_2)_2$; $\phi(CH_2)_4$; ϕCH_2· R = $\phi CH(OCOEt)CH_2CH_2$; $\phi CH{=}CHCH_2$; $\phi(CH_2)_3$; $\phi CH{=}CH(CH_2)_2$; CH_3. [i] R = $\phi(CH_2)_3$; $\phi(CH_2)_2$; $\phi(CH_2)_4$; ϕCH_2· R = $\phi CH(OAc)CH_2CH_2$; $\phi CH{=}CHCH_2$; $\phi(CH_2)_3$; $\phi(CH_2)_2$; $\phi CH{=}CH(CH_2)_2$; CH_3. [j] R = $\phi(CH_2)_3$; $\phi(CH_2)_2$; $\phi(CH_2)_4$; ϕCH_2· R = $\phi CH{=}CHCH_2$; $\phi(CH_2)_3$; $\phi(CH_2)_2$; $\phi CH{=}CH(CH_2)_2$; CH_3.

should cause, under steady-state conditions, proportionate variations of activity in both series. If a point is plotted whose abscissa is the logarithm of the activity for the appropriately substituted compound in one series and whose ordinate is the logarithm of the activity in an identically substituted compound in the second series, the resultant points should describe a straight line. Such a proportionality is known as a linear free energy relationship (1). Linearity would be a consequence of similar binding modes of two different analgesiophores[1] and would be due to the fact that the change in $\Delta S = 0$ or that variations in ΔS are proportional to changes in ΔH. If the modes of interaction are quite dissimilar, then identical *N*-substituents in two different series would experience a different physicochemical environment on the receptor. Such a situation would give rise to nonproportionate differences in ΔS which would be manifested by a nonparallel relationship in pharmacological activity. If the binding modes of two different analgesiophores containing identical substituents are similar, the slope of the regression should be in the vicinity of 1. This, of course, is dependent on the assumption that prior to the drug–receptor interaction, identical substituents on two different analgesiophores will affect the biodistribution of the compounds in a similar fashion. This assumption is quite reasonable in view of the successful application of substituent constants (2) for the purpose of predicting drug availability at the site of action. When identical substituents attached to different analgesiophores are not positioned with the same sites on the receptor, a scattering of points may make the value of the slope indeterminate. If an equilibrium mixture of dissimilar binding modes exists for each of two different analgesiophores, then it is possible that, depending on the composition of the mixture, degrees of point scattering may be observed with a slope which still approximates a value of 1. As differences in the modes of interaction increase, the standard deviation (S.D.) of points which compose the regression will become quite large and ultimately result in a nonparallel relationship.

To illustrate the existence of a linear free energy relationship, the author has used the data of Janssen and Eddy (3), who have reported on the analgesic activity of several series (I, II, and III) of identically substituted phenylpiperidine derivatives. It seemed appropriate to study these series because varying the *N*-substituent can cause large changes in analgesic potency. Moreover, their data are reported with well-defined confidence limits, and an additional advantage was that regressions obtained from the data of Janssen could be compared with those of Eddy.

The regressions have been compiled in Table I. Although the least-square values of slopes obtained from both Janssen's and Eddy's data represent a first approximation due to the

[1] The analgesic molecule less the substituent on the basic nitrogen.

limited number of compounds tested, it can be seen that the values obtained from two different sources are in good agreement and show high linear correlation coefficients (r). Significantly, the slopes of the regressions are fairly close to unity. This is consistent with the idea that at least a portion of the analgesiophore in each series may be interacting with the analgesic receptor in a similar manner and therefore contributing to the pharmacological effect by the same mechanism.

It is of interest to point out that there is no parallelism in analgesic activity between substituted phenylpiperidine derivatives and structures related to morphine (4–7), despite the fact that both meperidine and morphine are antagonized by nalorphine (8). This suggests that compounds in series I, II, and III and structures related to morphine are interacting with common receptors but that different molecular modes of binding occur. Such nonparallelism may be reflective of differences in the conformational and positional binding modes between substituted phenylpiperidines and morphine-like structures.

Such a method of comparing modes of molecular binding to common receptors conceivably can be applied to other types of medicinal agents.

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Books

REVIEWS

Pharmacotherapeutics of Oral Disease. Edited by A. H. KUTSCHER, E. V. ZEGARELLI, and G. A. HYMAN. McGraw-Hill Book Co., Inc., 330 W. 42nd St., New York, N. Y. 10036, 1964. xx + 690 pp. 18.5 × 25.5 cm. Price $17.50.

This is a specialized text to provide the student and practicing dentist with a usable reference on management and treatment of oral disease. Oral diagnosis is not included, although brief reviews of certain disease states are included. Emphasis is placed on the current treatments of various oral diseases and the therapeutic applications and dosages of many drugs. Attention also is given to systemic conditions which affect oral health or which must be given special consideration during dental procedures to emphasize systemic–oral interrelationships.

Part One summarizes the general principles of pharmacology, drug classification, federal laws, and the role of the American Dental Association in the evaluation of drugs.

Part Two presents discussions of drug groups based on pharmacological action.

Part Three covers the treatment of specific oral and dental diseases as well as certain systemic diseases.

An interesting feature of the book is the "Epitome" in which the editors have united pertinent information concerning the action, application, and untoward reactions of certain drugs used in many areas of dentistry. Included in the "Epitome" are analgesics, antibacterials, antibiotics, antihistamines, corticosteroids, hemostatics, local anesthetics, protectants, sedatives, tranquilizers, and vitamins. The "Epitome" is printed on tinted paper in the middle of the book for easy location. An 8-page chapter is devoted to the general subject of prescription writing.

British Pharmacopoeia 1963, Addendum 1964. General Medical Council. The Pharmaceutical Press, 17 Bloomsbury Square, London WC1, 1964. Price $5.40.

Under the direction of the General Medical Council, "The Addendum 1964" is published to present additions and/or deletions to the "British Pharmacopoeia 1963." Changes have been made in certain monographs and methods which are presently in the B. P. Attention is given to the incorporation of new developments into the procedures for detection of related foreign steroids by chromatographic analysis and for sterility testing. In addition, 60 monographs are presented for drugs and preparations which have not been described previously by the B. P. The "Addendum" became official on June 1, 1965.