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COMPETITIVE AND NON-COMPETITIVE INHIBITION OF BRADYKININ ON THE GUINEA-PIG ILEUM

BY

J. GARCIA LEME AND M. ROCHA E SILVA

From the Department of Pharmacology, Faculty of Medicine, U.S.P. Ribeirão Prêto, S. Paulo, Brasil

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In previous work (Rocha e Silva & Garcia Leme, 1963), we have investigated the anti-bradykinin effects of a series of compounds, following Mariani (1961) who found that chlorpromazine and phenergan inhibited the actions of bradykinin on the guinea-pig ileum.

These studies were later extended to other phenothiazine derivatives and structurally related substances, and to sympatholytic drugs (Rocha e Silva & Antonio, 1960). It was observed in the course of this work that tricyclic structures, such as those derived from phenothiazine, imipramine and cyproheptadine, produced a reversible antagonism for bradykinin, whereas with sympatholytic drugs the antagonism was irreversible. However, drugs that acted as reversible antagonists were also shown to act noncompetitively.

In the present paper the inhibitory actions of a large series of compounds, including dibenzazepine, thiaxanthene, cycloalkindole, dihydrodibenzocycloheptane and benzodiazepine derivatives, besides other phenothiazine, dihydrodibenzazepine and dibenzocycloheptene derivatives, are analysed in an attempt to find more potent and more specific antagonists to bradykinin.

METHODS

Assays were performed on the guinea-pig ileum preparation. A strip 2 to 3 cm long was suspended in 7 ml. of Tyrode solution at 37° C; contractions were recorded on a smoked drum through a frontal-writing lever. Doses were applied after 30 to 60 min.

At the beginning of the assay, responses were obtained to a series of three doses of the agonist; each dose was repeated two or three times. The drug to be tested as antagonist was then added to the nutrient stock solution, and was kept in continuous contact with the preparation. At the end of the assay the antagonist was removed by switching to the control Tyrode reservoir and recovery was tested. After each increase in the concentration of the antagonist the preparation was allowed to rest for 30 min and then the control series was repeated till constant responses were obtained; at this point the equilibrium between agonist and antagonist was usually achieved.

In all experiments a concentration of 10^{-7} g/ml. of atropine was kept in the bath, since atropine stabilizes the effects of bradykinin (Rocha e Silva & Garcia Leme, 1963).

Results were obtained from equilibrium responses after plotting the reciprocals of the effects (1/y) against the reciprocals of the doses (1/x), according to a procedure described by Rocha e Silva (1959, 1960, 1961). The calculation of the inhibitory potency of a drug was made by the pK_i method (Rocha e Silva, 1963; Rocha e Silva & Garcia Leme, 1963).

The pK_i, as an index of inhibition, refers to the concentration of the antagonist that doubles the value of β, this being the ratio of the slope of the line corresponding to a certain degree of inhibition and the slope

of the line corresponding to the control experiment in the double reciprocal plots. Thus the value of β indicates the factor by which the slope of the control line is multiplied after the addition of the antagonist to the bath.

Noncompetitive inhibition follows Schild's (1954) equation. With the obvious transformations, and taking the reciprocal of the effects and of the concentrations, it can be put into the more familiar form:

$$1/y = \beta K/x + \beta/y_m$$

where $\beta = 1 + I/K_i$ or $1 + (I)^n/(K_i)^n$, referring to a mono- or a multimolecular antagonism respectively, $1/y_m$ indicates the intercept of the line with the 1/y axis, I is the concentration of the inhibitor and K_i the apparent constant of dissociation of the antagonist with the receptors.

When $(I) = K_i$, $\beta = 2$.

The values of pK_i can be deduced from the equation:

$$\log (\beta - 1) = n [pK_i - p(I)],$$

where $n = 0.95/(pA_2 - pA_{10})$ or, graphically, as the reciprocal of the log of the concentration of the inhibitor which doubles the slope of the control line ($\beta = 2$).

It seems clear that, for a competitive antagonism, $pK_i = pA_2$, but for a noncompetitive antagonism the concentration of the inhibitor necessary to double the slope is generally higher than that necessary to reduce the effect of a double dose to that produced by a single one (Fig. 1), which corresponds to a double slope in the competitive inhibition.

The potencies of competitive and noncompetitive antagonists shown in Table 1 are the reciprocals of the log of the concentrations which give $\beta = 2$, or the values of pK_i. In most cases, the simplified graphical method was applied. The pK_i values are calculated in w/v. To obtain pK_i (M) it is necessary to add $\log_{10}$ of the molecular weight (M.W.) $\times 10^{-3}$ or:

$$pK_i \text{ (M)} = pK_i \text{ (w/v)} + \log_{10} (\text{M.W.} \times 10^{-3})$$

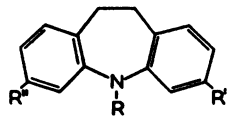
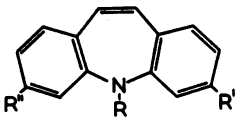
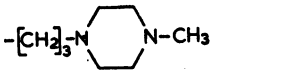
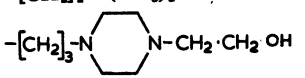
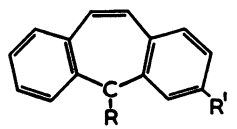
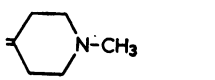
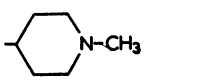
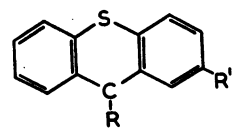
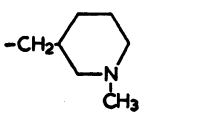
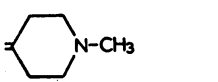
All pK_i values were obtained from at least two experiments.

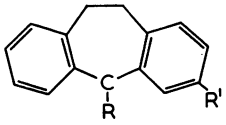
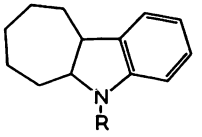
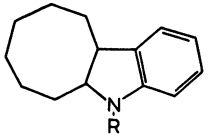
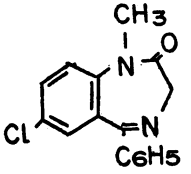
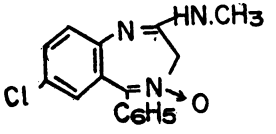
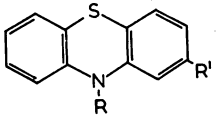
The drugs used were: imipramine [5-(3-dimethylaminopropyl)-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine] hydrochloride; 3,7-dichloro-5-(3-dimethylaminopropyl)-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine (G-28364); 5-(2-dimethylaminopropyl)-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine hydrochloride (G-22150); 3,7-dichloro-5-(3-dimethylaminopropyl)-5*H*-dibenz[*b*,*f*]azepine (G-31515); 5-[3-(4-methylpiperazin-1-yl)propyl]-5*H*-dibenz[*b*,*f*]azepine hydrochloride (G-32052); 5-(2-dimethylaminoethyl)-5*H*-dibenz[*b*,*f*]azepine fumarate (G-31406); opipramol (5-{3-[4-(2-hydroxyethyl)piperazin-1-yl]propyl}-5*H*-dibenz[*b*,*f*]azepine) hydrochloride (Insidon, J. R. Geigy A.G., Switzerland); cyproheptadine [4-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine] hydrochloride; 4-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)-1-methylpiperidine maleate (L-583232); dihydrochlorprothixene [2-chloro-9-(3-dimethylaminopropyl)thiaxanthen] hydrochloride (L-290698); 2-chloro-9-(1-methylpiperid-4-ylidene)thiaxanthen maleate (L-581512); 3-chloro-5-(3-dimethylaminopropyl)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene hydrochloride (L-572451); amitriptyline [5-(3-dimethylaminopropylidene)-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene] hydrochloride (Merck Sharp & Dohme, U.S.A.); methixene [9-(1-methylpiperid-3-ylmethyl)thiaxanthen] hydrochloride (Tremaril, Wander, Brazil); 5-(3-dimethylaminopropyl)-5,6,7,8,9,10-hexahydrocyclohept[*b*]indole hydrochloride (Wy 1611A); 5-(dimethylaminomethyl)-6,7,8,9,10,11-hexahydrocyclo-oct[*b*]indole hydrochloride (Wy 3263); 7-chloro-1,3-dihydro-1-methyl-2-oxo-5-phenyl-2*H*-1,4-benzodiazepine (Wy 3467); promazine 10-(3-dimethylaminopropyl)phenothiazine] hydrochloride; chlorpromethazine [2-chloro-10-(2-dimethylaminopropyl)phenothiazine] hydrochloride (Chlorphenergan, Wyeth Lab., U.S.A.); promethazine [10-(2-dimethylaminopropyl)phenothiazine] hydrochloride (Phenergan); trimipramine [5-(3-dimethylamino-2-methylpropyl)-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepine] hydrochloride (Surmontil, Rhodia, Brazil); and chlordiazepoxide [7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine-4-oxide] (Sintofarma, Brazil). In all experiments, synthetic bradykinin (BRS 640, Sandoz, Switzerland) was used as agonist. Solutions were in distilled water; when free bases were employed, the equivalent amount of acid was added to dissolve the material.

TABLE 1

CHEMICAL CONFIGURATION AND INHIBITORY POTENCY OF BRADYKININ ANTAGONISTS

NC refers to noncompetitive inhibition, C to competitive inhibition

Compound	Form	Nucleus	Substitution groups R	R′	R″	pK_i values	Type of antagonism
I, Dihydrodibenzazepine derivatives							
Imipramine	Hydrochloride		$-[CH_2]_3.N(CH_3)_2$	-H	-H	6·10–6·40	NC
Trimiprimine	Hydrochloride		$-CH_2.CH(CH_3).CH_2.N(CH_3)_2$	-H	-H	6·30	NC
G–28364	Free base		$-[CH_2]_3.N(CH_3)_2$	-Cl	-Cl	6·30–6·40	NC
G–22150	Hydrochloride		$-CH_2.CH(CH_3).N(CH_3)_2$	-H	-H	5·85–5·95	NC
II, Dibenzazepine derivatives							
G–31515	Free base		$-[CH_2]_3.N(CH_3)_2$	-Cl	-Cl	6·70–7·00	NC
G–32052	Hydrochloride			-H	-H	7·00	NC
G–31406	Fumarate		$-[CH_2]_3.N(CH_3)_2$	-H	-H	6·20–6·50	NC
Opipramol	Hydrochloride			-H	-H	5·50	NC
III, Dibenzocycloheptene derivatives							
Cyproheptadine	Hydrochloride			-H	—	7·00–7·30	NC
L–583232	Maleate			-H	—	7·40–7·50	C
IV, Thiaxanthene derivatives							
Methixene	Hydrochloride			-H	—	6·60–6·80	C
Dihydrochlorprothixene	Hydrochloride		$-[CH_2]_3.N(CH_3)_2$	-Cl	—	7·00–7·30	C
L–581512	Maleate			-Cl	—	6·20-6·40	NC

V, Dihydrodibenzocycloheptene							
L-572451	Hydrochloride		$-[CH_2]_3.N(CH_3)_2$	$-Cl$	—	6·20	NC
Amitriptyline	Hydrochloride		$=CH.[CH_2]_2.N(CH_3)_2$	$-H$	—	6·10	NC
VI, Cycloalkindole derivatives							
Wy 1611A	Hydrochloride		$-[CH_2]_3.N(CH_3)_2$	—	—	5·50	NC
Wy 3263	Hydrochloride		$-CH_2.N(CH_3)_2$	—	—	5·80	NC
VII, Benzodiazepine derivatives							
Wy 3467	Free base		—	--	—	5·50	NC
Chlordiazepoxide	Free base		—	—	—	No inhibition	—
VIII, Phenothiazine derivatives							
Promazine	Hydrochloride		$-[CH_2]_3.N(CH_3)_2$	$-H$	—	5·80	NC
Chlorpromethazine	Hydrochloride		$-CH_2.CH(CH_3).N(CH_3)_2$	$-Cl$	—	6·50–6·60	NC
Promethazine	Hydrochloride		$-CH_2.CH(CH_3).N(CH_3)_2$	$-H$	—	5·30	NC

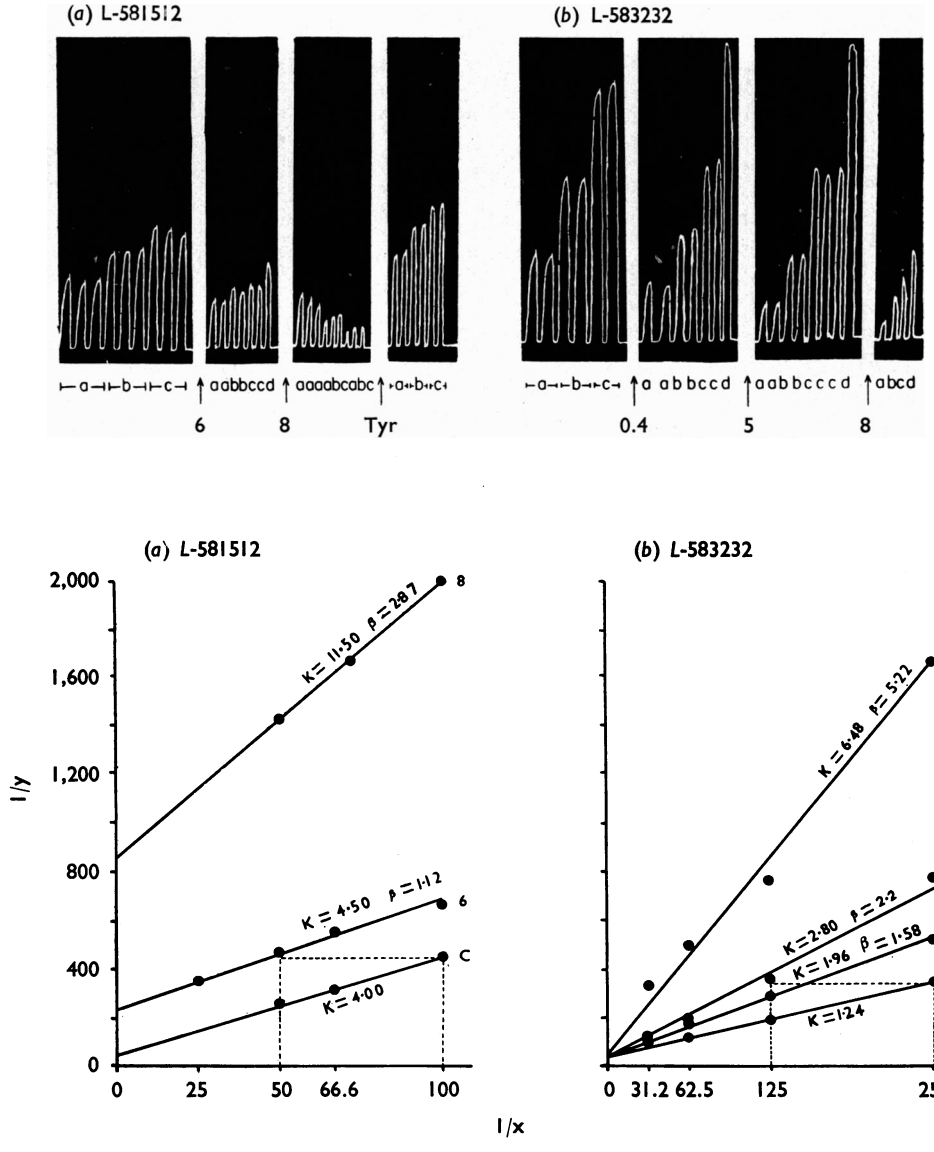


Fig. 1. Kymograph recordings and corresponding double reciprocal plots of two experiments in which L-581512 (*a*) and L-583232 (*b*) were used as antagonists of bradykinin. Doses of bradykinin are: a 0.1, b 0.15, c 0.2 and d 0.4 μg for L-581512; and a 0.04, b 0.08, c 0.16 and d 0.32 μg for L-583232. Antagonists were given at the arrows, concentrations in g/ml. $\times 10^{-7}$. Tyr=Tyrode solution. Different intercepts over the 1/y axis are observed in the noncompetitive inhibition (experiment with L-581512) and a common intercept in the competitive antagonism (experiment with L-583232), when reciprocals of the effects (1/y) are plotted against the reciprocals of the doses (1/x). Observe that in the noncompetitive inhibition the concentration of the antagonist (values on right of graphs, in 10^{-7} g/ml.) which reduces the effect of a double dose to that produced by a single one does not double the slope of the corresponding line, as indicated by the dotted lines. K=Slope of the lines; β=ratio of the slopes; C=control.

RESULTS

The compounds tested can be classified into the following groups according to their chemical structures, whose relationship and inhibitory potencies (pK_i values) are indicated in Table 1.

I, Dihydrodibenzazepine derivatives. All compounds of this group acted as non-competitive inhibitors, with pK_i values of the same order, G-22150 being somewhat less potent than its congeners.

II, Dibenzazepine derivatives. Like the drugs of Group I, these compounds produced noncompetitive antagonism, being, however, more potent.

III, Dibenzocycloheptene derivatives. More potent than the compounds listed above, cyproheptadine was a noncompetitive antagonist, while L-583232 produced a competitive type of inhibition.

IV, Thiaxanthene derivatives. Methixene and dihydrochlorprothixene inhibited competitively the actions of bradykinin; L-581512 inhibited it noncompetitively.

V, Dihydrodibenzocycloheptene derivatives. L-572451 and amytriptyline produced a mild, noncompetitive type of inhibition.

VI, Cycloalkindole derivatives. Wy 1611A, related to cyclohept[*b*]indole, and Wy 3263, related to cyclo-oct[*b*]indole, induced a weak, noncompetitive antagonism against bradykinin.

VII, Benzodiazepine derivatives. Weak, noncompetitive inhibition was attained with Wy 3467, but chlordiazepoxide concentrations of 2×10^{-6} were unable to antagonize bradykinin.

VIII, Phenothiazine derivatives. Promazine, chlorpromethazine and promethazine are noncompetitive and weak inhibitors. Larger series of phenothiazine drugs have been previously assayed (Rocha e Silva & Garcia Leme, 1963).

Figs. 1 and 2 show graphical representations of competitive and noncompetitive inhibition of bradykinin, produced by the addition of L-583232, L-581512, methixene and chlorpromethazine to the bath.

DISCUSSION

Our results suggest that particular chemical configurations are necessary for competitive inhibition of bradykinin effects on the guinea-pig ileum.

Comparing the structures shown in Fig. 3 and the results listed in Table 1, the presence of either a seven-carbon ring or a six-membered ring containing one sulphur atom in the tricyclic structure of the substances tested seems important for competitive antagonism.

Tricyclic structures with modifications in the central ring of the nucleus, as in the cycloalkindoles, and benzodiazepine derivatives have been shown to induce very weak, noncompetitive inhibition or no inhibition at all. With tricyclic structures containing a seven-carbon ring, a double bond between C_{10} and C_{11} seems to be necessary for competitive antagonism. When these double-bonded carbon atoms are replaced by a sulphur atom (–S–) which is isosteric with the former –C=C–, competitive antagonism is maintained, as can be seen by comparing the inhibitions by L-583232, methixene and dihydrochlorpro-

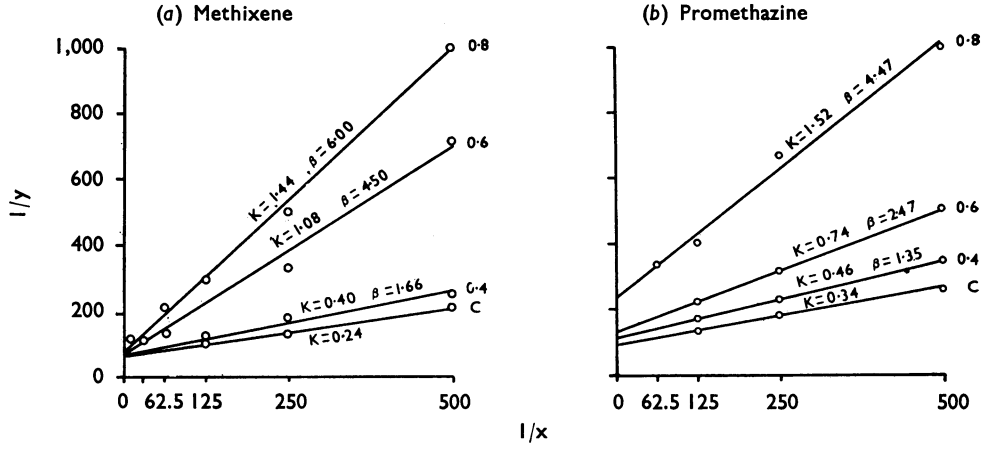


Fig. 2. Double reciprocals plots of experiments in which methixene (*a*) and chlorpromethazine (*b*) were used as antagonists of bradykinin. 1/y refers to the reciprocals of the effects and 1/x to the reciprocals of the doses. Symbols as in Fig. 1. Concentrations in g/ml. × 10^{-6}.

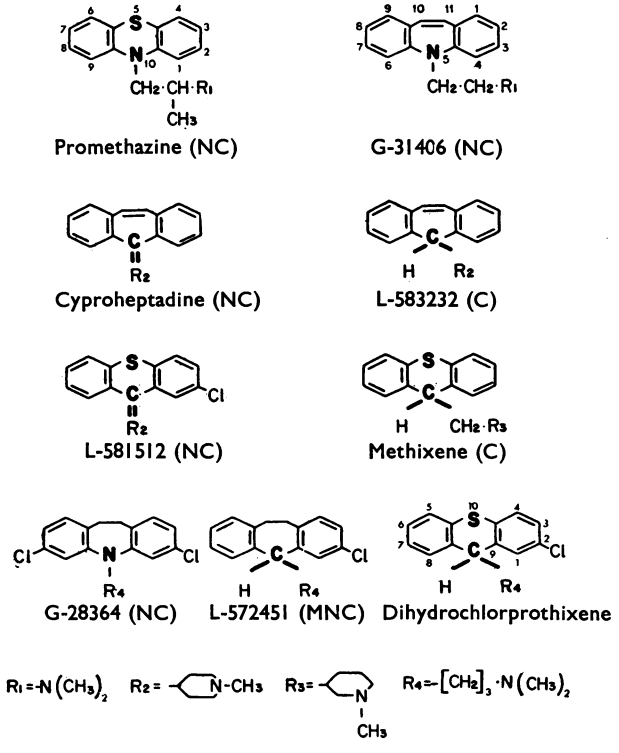


Fig. 3. Chemical structures of some substances tested as antagonists of bradykinin to show the main differences in the molecular configurations observed in competitive (C) and noncompetitive (NC) inhibitors. See text.

thixene. However, the heterocyclic character of the central ring seems to be compatible with competitive action only when there is isosterism of the type –S– equivalent to –C=C–, since the introduction of a nitrogen atom in position 5 of the tricyclic structures containing a seven-membered central ring makes the molecule noncompetitive in its antagonism towards bradykinin. The same seems to be true for tricyclic structures containing a six-membered sulphur-containing central ring, since replacement of the carbon atom carrying the side-chain by a nitrogen atom does not maintain a competitive type of inhibition. This can be seen by comparing the inhibitions by dihydrochlorprothixene and promethazine. A similar difference occurs with tricyclic structures containing a seven-membered central ring, as is seen from comparison of the inhibitions by L-583232 and that with G-31406.

Another point to be emphasized about persistence of competition is the importance of the lack of a double bond in the attachment of the side-chain to the central nucleus. Thus, L-583232 produced competitive inhibition, but the closely related compound, cyproheptadine, which differs only in the presence of a double bond in the attachment of the side-chain, acts as a noncompetitive inhibitor. A similar comparison can be made between methixene (competitive) and L-581512 (noncompetitive inhibition).

Modifications in the side-chain or minor substitutions in the nucleus, as, for example, the introduction of chlorine atoms or methyl groups, do not seem to be of great importance in the enhancement of the inhibitory potencies or upon the nature of the antagonism.

Most of the compounds tested might not be sufficiently potent to serve for practical purposes. However, careful and extensive trials have to be done *in vivo* to test the real value of these drugs in circumstances in which release of bradykinin might be concerned. With some of them a correlation between antagonistic potency and reduction of the thermic oedema in the rat's paw has been observed.

It can be argued whether the inhibition observed would correspond to a nonspecific action of these substances upon the isolated smooth muscle. Though this can be true concerning some of the drugs classified as noncompetitive inhibitors, it would be difficult to sustain such an opinion when competitive antagonists are concerned.

SUMMARY

1. The actions of a large series of compounds including dibenzazepine, thiaxanthene, cycloalkindole, dihydrodibenzocycloheptene, benzodiazepine, dihydrodibenzazepine, dibenzocycloheptene and phenothiazine derivatives on the effects of bradykinin on the guinea-pig ileum preparations were analysed in the attempt to find an antibradykinin agent.

2. Most of the substances acted as noncompetitive inhibitors but some thiaxanthene and dibenzocycloheptene derivatives produced a competitive antagonism against bradykinin.

3. Analysis of the structure of the compounds tested suggests that particular chemical configurations are responsible for the competitive character of the inhibition.

We are greatly indebted to Geigy, Merck Sharp & Dohme, Wyeth, Rhodia, Wander and Sintofarma Laboratories for the supply of the drugs; to Drs E. Stürmer and C. Cerletti (Sandoz, Basel) for the generous gift of synthetic bradykinin; and to Professor Q. Mingoia for his valuable suggestions.

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