

CUMULATIVE DOSE-RESPONSE CURVES

I. Introduction to the technique

BY

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INTRODUCTION

The study of drugs in isolated organs was greatly stimulated by Rudolph MAGNUS more than half a century ago. The advantage of pharmacological studies in isolated organs over those on intact animals is the simplicity of the response and the fact that the concentration of the drug near the locus of action is directly related to the dose administered, since transport and drug metabolism hardly come into the picture.

The technique generally used is that dose-response curves of stimulant drugs (agonists) are made by measuring the response of the isolated organ on single doses. In many cases this response remains constant as long as the concentration of the drug in the bath fluid does not alter. In exceptional cases when a single dose is given the response first reaches a maximum and then decreases to a steady value (fading). When stable drugs are used and if fading is not involved, it is not necessary to evaluate the responses of a number of individual doses in order to obtain a dose-response curve. Cumulative dose-response curves can then be made by increasing the concentration of the drug in the bath fluid step by step without washing out after each single dose. This cumulative technique is quite simple and requires a fraction of the time needed for the individual dose technique. As yet the cumulative procedure is not generally used, although it has extensively been applied by Aulfs *et al.* in studies

on molecular pharmacology (for references see part II). It is the aim of the present paper to break a lance for this technique. Furthermore we will demonstrate how easily sets of curves are obtained in this way, which curves are of basic importance for studying the mechanism of action of new compounds.

METHODS AND RESULTS

The isolated intestine (ileum or jejunum) of rats and guinea-pigs are most frequently used for pharmacological studies *in vitro*. In the present paper we only deal with the technique of making cumulative dose-response curves in the rat and guinea-pig intestine, while a detailed description of the technique in other isolated organs will be given in a subsequent paper.

Pieces of 1.5-2 cm of intestine are suspended in tyrode solution kept at 37° centigrade. Composition of tyrode: 1 L aqua bidest.; 8.0 g NaCl; 0.2 g KCl; 0.2 g CaCl₂; 0.1 g MgCl₂; 1.0 g NaHCO₃; 0.05 g NaH₂PO₄ and 1 g glucose.

The intestine is attached to a lightly loaded isotonic lever. The magnification by the lever is about 20 times while it is adjusted so that the maximum response actually to be recorded with the intestine does not exceed 20 cm.

When the intestine has been in the bath for about 10 min., having in the meantime been washed several times, the volume of the bath fluid is adjusted to 10 ml. Then a stimulant drug (spasmogen) is introduced into the bath in cumulative amounts, using the sequence 1, + 2, + 7, + 20, + 70, + 200 etc. so that the drug concentration in the bath increases stepwise in the series 1; 3; 10; 30; 100; 300 etc. Concomitantly with the increase in concentration the contraction of the gut increases step by step. If the next concentration step does not cause a further increase in contraction, it is assumed that the maximum effect for that particular drug is obtained and the drug is washed out. The time required for making a complete curve is in general not more than 2 minutes. After washing for 2-5 minutes the next curve can be made. In this way, in one piece of intestine 10-30 curves can be obtained in 2-5 hours.

Records of cumulative dose-response curves of histamine-like and muscarine-like drugs are presented in Figure 1. It should be noted that the next dose is introduced into the bath when equilibrium for the preceding dose is reached. Histamine (H) is used as a reference drug for the histamine-like drugs studied in the guinea-pig jejunum and

furtherthonium (HFur) as a standard of parasympathomimetics in the rat intestine. Cumulative dose-response curves of the reference compound are made alternatively with those of the other stimulants. It may be observed from these records that mecholyl (MeCh), carbamylcholine (CarbCh), reversed acetylcholine (rACh) and choline acquire maximum heights identical with the reference compound furtherthonium, although quite different doses are needed in order to obtain a 50% effect. Similarly

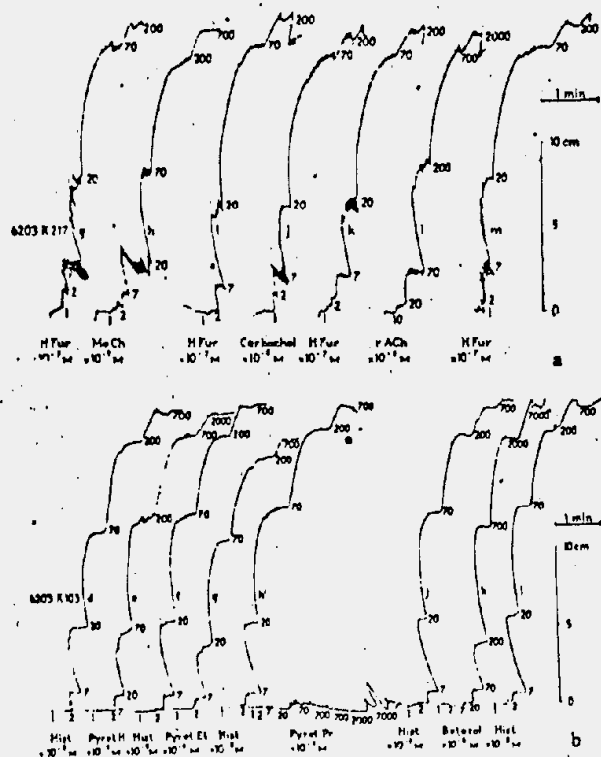


FIG. 1

Kymographion records of the response of the guinea-pig intestine (a) and rat intestine (b) to various stimulant drugs. For each cumulative dose-response curve the doses are increased stepwise in a geometric sequence. The next dose is introduced when the preceding one has reached a steady value. When the next dose does not cause a further contraction the intestine is washed out. The time scale in the figures applies for the individual curves. During washing the drum is stopped and turned back to some degree in order to save paper. The lever is in horizontal position in the midst of the drum. This implies that the curve is started with the lever downwards. It therefore seems as if at low doses the time effect record goes in the wrong direction. Obviously with a frontal lever this would not be the case.

Note that a cumulative dose-response curve can be made in about 2 minutes.

the maximum height of the records of the histamine-like drugs pyrethamine (PyretH) and betazol is identical with that of histamine whereas the equipotent doses are largely different. The maximum height of the

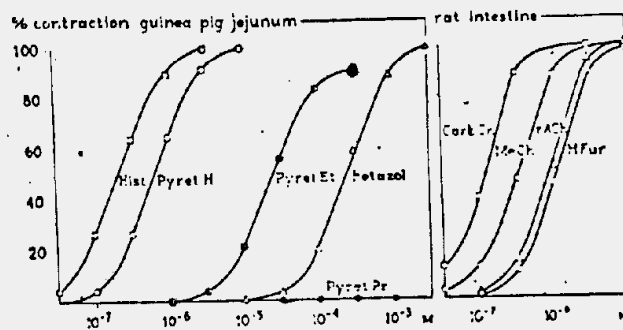


FIG. 2

Dose-response curves as calculated from the records of figure 1.

The maximum responses of the reference drugs are fixed at 100 percent and the effect of each cumulative concentration is expressed in percentages of the maximum height of the preceding curve of the standard.

Note a difference in potency as a difference in the position of the curves on the dose-axis. Note further that the N-Ethyl derivative of pyrethamine is only partially agonistic (maximum height is about 80% of that of histamine).

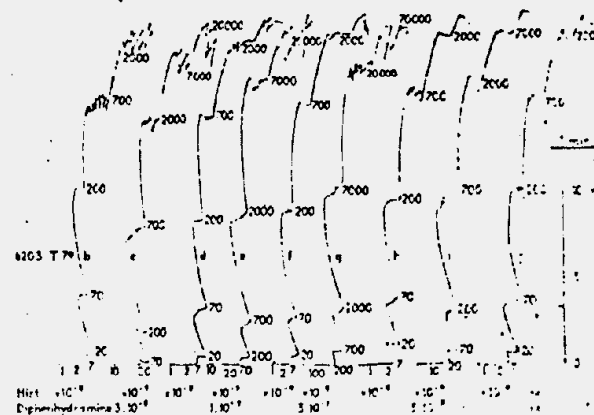


FIG. 3

Kymographion records of the guinea-pig intestine to histamine both in the presence and absence of diphenhydramine. The cumulative dose-response curves of histamine are made in the same way as those for the stimulants in figure 1.

Note that the curves remain identical in shape but that in the presence of diphenhydramine higher doses are needed in order to obtain the same response (antagonism). Diphenhydramine is a non-stimulant drug and therefore inactive of its own.

N-ethyl derivative of pyrethamine (PyretEt) is about 80% of that of histamine so that this compound must be regarded as a partial agonist.

From the records of Figure 2 dose-response curves are obtained by expressing the effect of each cumulative dose in a percentage of the maximum response of the preceding curve of the reference drug. The percentage effect is plotted versus the logarithm of the dose. See Figure 2.

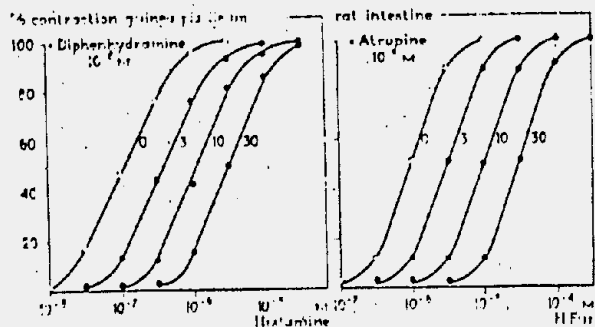


FIG. 4

Dose-response curves for histamine in the guinea-pig intestine as calculated from the records of figure 3 both in the presence and absence of diphenhydramine (a) and for furthrethonium in the rat intestine in the presence and absence of atropine (b).

Note that the curves of the stimulant drugs are shifted to higher concentrations by the antagonist whereas the maximum effect is not altered. This implies a competitive antagonism between histamine and diphenhydramine and between furthrethonium and atropine.

The curves of Figure 1a and 2a as well as Figure 1b and 2b are made in a single isolated organ. The responses of such a single organ remain reasonably constant for hours, as may be seen from the curves of the reference drugs (FIG. 1). Organs of various animals usually differ to a greater degree. With the individual dose technique various organs are needed in order to make a set of curves like those of Figure 2. Minor differences in dose-response curves can therefore be found more easily when using the cumulative dose-technique.

Dose-response curves of stimulant drugs or agonists are characterized by the maximum height, the slope of the curve and its position on the dose-axis.

DISCUSSION

Since drugs are chemical substances the primary feature of any type of drug action is the reaction of drug molecules with molecules in the

tissue. In other words a reaction of micromolecules with parts of macromolecular substances. The reactive sites in the tissue are called receptors. The tendency of a drug to occupy receptors has been termed the *affinity*. The affinity alone is not sufficient for a drug to act as a stimulant, that is, different drugs which occupy certain receptors may differ in their ability to cause an effect. As a matter of fact drugs may exist that merely occupy receptors without eliciting a response. The ability to generate an effect once receptors become occupied has been termed *intrinsic activity* (1, 2) or *efficacy* (4).

Pure agonists, as for instance acetylcholine and furthrethonium, histamine and betazol, have an intrinsic activity equal to unity, since their maximum effect is equal to the maximum response obtainable with the organ.

A partial agonist, as for instance PyretEt as a histaminic, and pilocarpine as a parasympathomimetic (3), has intermediate intrinsic activity ($0 < i.a. < 1$).

Inactive drugs may have an intrinsic activity equal to zero. As a matter of fact inactive drugs may either have no affinity or no intrinsic activity. Drugs with affinity but zero intrinsic activity occupy receptors in certain doses and therefore may interfere with receptor occupation of the stimulant drug. Consequently they may cause an inhibition. Since both the stimulant and the "inactive" drug occupy the same receptors such an inhibition is obviously of a competitive nature.

The mechanism of action of inactive drugs can rapidly be investigated with the cumulative dose-response curve procedure. For this purpose dose-response curves are made for an agonist both in the presence and absence of various doses of the inactive substance. Records for the agonist histamine in the presence of diphenhydramine, which is inactive by nature are presented in Figure 3. It may be seen that the curves of histamine remain identical in shape but in the presence of diphenhydramine larger doses are needed in order to get the same response. However, the maximal response with histamine can always be obtained by increasing the dose. From such records in a single piece of intestine a family of curves can be calculated. See Figure 4a. In Figure 4b a family of dose-response curves is given for furthrethonium and the parasympatholytic atropine which are obtained on a single piece of rat intestine. A competition should be noted in both cases of Figure 4 as a parallel shift of the curves of the agonist.

In order to obtain a picture like those in Figure 4 quite a large amount of work would be entailed when using the single dose-technique since in that case different organs are needed and these organs differ in sensitivity.

It goes without saying that once the mechanism of action of a drug has been clarified, experiments on various organs are required in order to get average data for the drug parameters. Methods for the calculation of drug parameters from dose-response curves and families of dose-response curves are given in part II.

SUMMARY

The technique is described of making cumulative dose-response curves for stimulant drugs in the isolated intestine.

Histaminic and muscarinic drugs have been used as stimulants.

The significance of the cumulative dose-response curve technique for the clarification of the mechanism of drug action is discussed.

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