Textbook of *in vitro* Practical Pharmacology

Ian Kitchen

C3866
R965-43
E9

Textbook of *in vitro* Practical Pharmacology

Ian Kitchen
BSc, PhD
Lecturer in Pharmacology
Department of Biochemistry
University of Surrey

BLACKWELL SCIENTIFIC PUBLICATIONS
OXFORD LONDON EDINBURGH
BOSTON MELBOURNE

© 1984 by Blackwell Scientific Publications
Editorial offices:
Osney Mead, Oxford, OX2 0EL
8 John Street, London, WC1N 2ES
9 Forrest Road, Edinburgh, EH1 2QH
52 Beacon Street, Boston, Massachusetts 02108, USA
99 Barry Street, Carlton, Victoria 3053, Australia

All rights reserved. No part of this publication may be reporduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior permission of the copyright owner

First published 1984

Set, printed and bound in Great Britain by Butler & Tanner Ltd
Frome and London

DISTRIBUTORS

USA
Blackwell Mosby Book Distributors
11830 Westline Industrial Drive,
St Louis, Missouri 63141

Canada
Blackwell Mosby Book Distributors
120 Melford Drive, Scarborough,
Ontario, M1B 2X4

Australia
Blackwell Scientific Book Distributors
31 Advantage Road, Highett,
Victoria 3190

British Library
Cataloguing in Publication Data

Kitchen, Ian
Textbook of in vitro practical pharmacology.
1. Pharmacology—Laboratory manuals
I. Title
615'.1'028 RM303

ISBN 0-632-01216-1

Contents

此为试读,需要完整PDF请访问: www.ertongbook.com

Preface

The most recent textbook covering experiments on isolated preparations was published over thirteen years ago. Since that time a great deal has happened in the field of *in vitro* practical pharmacology which has led me to write this new textbook. The advent of several new isolated tissue preparations in pharmacological research has filtered through to undergraduate teaching of pharmacology. The book therefore includes important additions to the traditional isolated tissues, such as the vas deferens, the ano-coccygeus and the taenia caeci muscles. In addition, the last decade has seen enormous growth in the use of electronic pharmacological recorders and transducers in the undergraduate teaching laboratory. Accordingly, the book contains an introductory section which includes details of operation of this relatively new teaching equipment. I hope this will be of value not only to the undergraduate pharmacologist but also to postgraduates who embark on research with isolated tissues and have only limited experience in this field. The postgraduate using isolated tissues for the first time may also find the fault-finding section helpful!

Each section of experiments is preceded by tabulated data of experimental parameters and dosages of drugs used. I hope these will be particularly useful for technicians involved in the setting up of practical classes and also as a quick reference source for lecturers involved in practical teaching.

Whilst the use of *in vivo* experiments for teaching pharmacology has been declining in recent years on academic, financial and ethical grounds, *in vitro* practical pharmacology is being retained and often expanded, with the possible exception of the pharmacology taught to medical students. Many medical colleges are now moving towards the use of videotaped practicals. Whilst I feel this is a useful medium, and one which I use myself, there is no substitute for some of the experiments included in this book. For example, the chance to see a fibrillating heart, first hand, is invaluable to the student of medicine. There is of course also the danger of drug response becoming stereotyped. Those involved in the teaching of *in vitro* and *in vivo* pharmacology know only too well that there is no such thing as a 'typical' drug response. This book is therefore aimed at all students who participate in practical pharmacology including medics, pharmacists and pharmacologists.

Three experiments in this book (Expt. 1, 4.7 and 6.6) have preliminary procedures which involve injections of drugs. These procedures require a Home Office Licence in the United Kingdom and must only be carried out by a Home Office Licence holder, covered by the appropriate certificates.

I have compiled this book from my involvement in the teaching of practical pharmacology at Chelsea College, North East London Polytechnic, two London medical schools (St Mary's and The London) and currently at the University of Surrey. I am grateful to the staff of these institutions for their help in the writing of the book and for the use of some of their teaching schedules. Each series of experiments has, where appropriate, photographic illustrations to aid dissection of tissues and also traces of typical responses of each preparation. I would like to thank John Darbey (AVA Unit, Surrey) for his expert photographic work, Paul Green for his assistance in setting up preparations and Dr Margaret Orchard and Julia McDowell for providing me with some of the traces.

The photographs of transducers, electrodes, stimulators and recording apparatus were provided by Harvard Apparatus, Grass Instruments and Ormed. I am grateful for their interest and help.

I would also like to express my thanks to Dr David Burleigh at The London Hospital Medical College for the criticisms and corrections of the manuscript and to Mrs Valerie Saunders for typing countless drafts. In connection with this I perhaps should add my thanks to the word processor. This book would not have been written without it.

Finally, I hope that the errors and omissions are few. I would welcome any comments or criticisms.

1983 I.K.

Introduction

1 General methodology

The methods employed in the use of isolated tissue preparations in practical pharmacology have changed somewhat in recent years, due to the introduction of more sophisticated equipment. It is becoming less common to see the traditional set-up of tissue baths, immersed in water contained within a perspex outer bath and heated by an open water circulator. In its place, both warming coils for the physiological salt solutions and the tissue baths have outer chambers and are heated by water circulators in a closed circuit. It is also clear that the days of the pivoted lever writing on a Kymograph drum, for recording movements of isolated tissues, are numbered. Details of the levers used in pharmacological experiments may be found elsewhere (Edinburgh University Pharmacology Staff, 1968). Many University and Polytechnic departments are now employing isotonic and isometric transducers coupled to an appropriate amplifier and recorder and the use of such equipment will undoubtedly increase. A detailed description of these pharmacological recorders and their operation is provided in section 3.

The viability of isolated tissues varies enormously. Some are extremely robust, are tolerant to over zealous handling and can withstand long periods without oxygen. Others, especially nervous tissue and cardiac muscle, require extremely careful manipulation and will not remain viable for long in the absence of oxygen. In general, it is best to treat all isolated preparations with respect, avoiding stretching of the tissue. Careful attachment to the tissue holder and correct loading of the transducer to give the recommended resting tension are also important. The use of dissecting instruments, especially forceps, should be kept to a minimum, and fingers should be used where possible. It is also worthy of note that ink used in recording apparatus is particularly toxic to many tissues.

2 Experimental parameters

Each section of experiments in this book is preceded by a summary of experimental parameters and drugs used in the experiments. The following sections give more detailed elucidation of each of these parameters.

2.1 Organ baths

A guide to the internal volume of organ (or tissue) baths required for each *in vitro* preparation is given throughout the book. For the majority of tissues this is not critical. There are, however, a few exceptions. Where transmural or field stimulation is to be used the dimensions of the organ bath will have an effect on the current passed and it is important not to exceed the recommended volume. The converse is true in experiments where additional apparatus is needed alongside the tissue (e.g. Finkleman preparation, Expt. 2.4). In this case the recommended volumes should not be reduced or the tissue will not be free to move within the bath.

The style of organ bath varies slightly from laboratory to laboratory, but basically consists of an internal chamber with an inflow and outflow for physiological salt solutions and a scinter glass at its base to allow aeration. This is jacketed by an outer warming jacket. The salt solution is fed through a coil which is also contained in a warming jacket. This coil is sometimes integrated within the outer chamber of the organ bath (Fig. 1).

The tissue is secured within the bath either on an internal hook located at the base or on a removable tissue holder. Where there is an internal hook the tissue must be attached by means of a loop tied at one end. The tissue holder has the advantage that the preparation can be securely tied to the hook on the holder's tip. Some purists would claim that only loops are acceptable as this allows the tissue total freedom of movement. Experience has indicated to the author that there is no difference whether loops or ties are used. The author favours the latter since loops have a habit of working free just at a crucial moment.

The need for washing of preparations varies and special instructions related to this are given in individual experiments. Some preparations are more suitably washed by overflow than drainage. This especially includes certain nerve muscle preparations and those muscles which exhibit spontaneous activity when exposed to air. In general a thorough wash should be given before equilibration, and regular washout periods should be maintained throughout the experimental period. Apparatus should always be washed with distilled water after use to prevent the growth of micro-organisms, and periodically cleaned with hydrochloric or chromic acid.

此为试读,需要完整PDF请访问: www.ertongbook.com

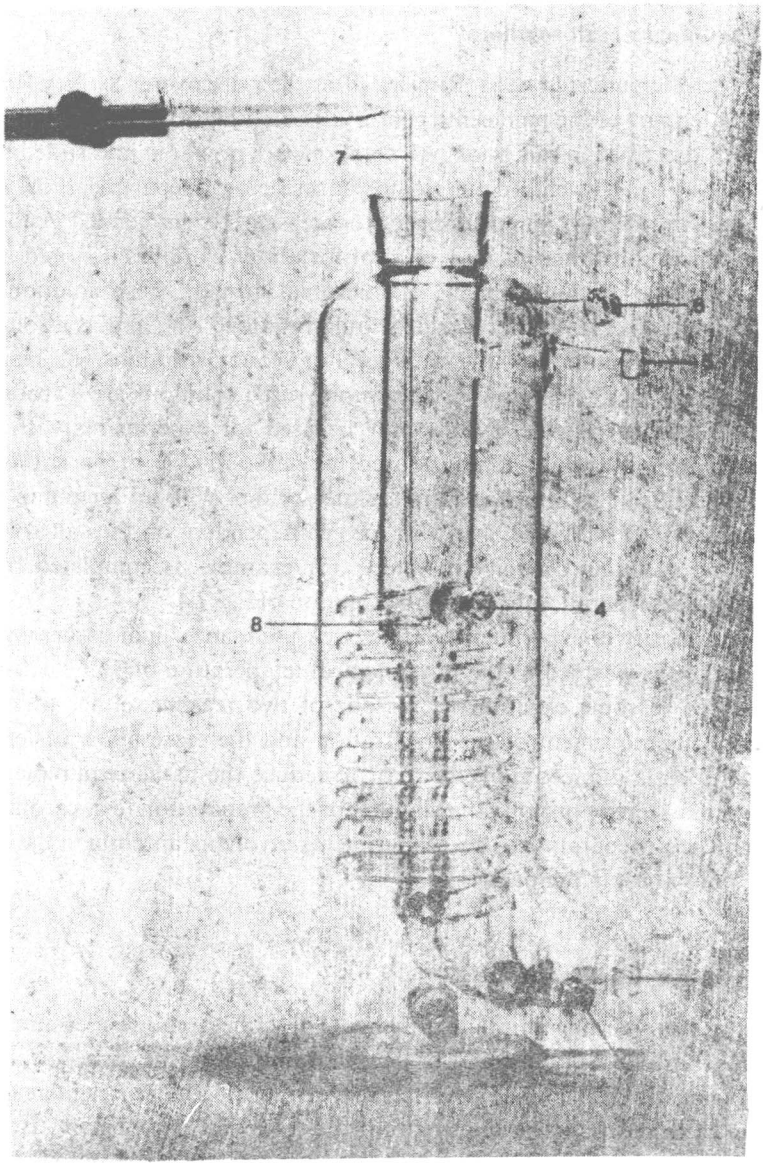

Fig. 1. Typical integrated coil organ bath.

1 Water circulator inflow for outer bath
2 Aeration inflow for inner bath
3 Ringer outflow
4 Ringer inflow
5 Ringer outflow for washing by overflow
6 Water circulator outflow for outer bath
7 Tissue holder
8 Scinter for aeration of inner bath

2.2 Physiological salt solutions

These are often referred to as 'Ringers' after their discoverer Sydney Ringer, in the latter part of the nineteenth century. Table 1 gives the formulae for the solutions described in this book. All solutions are prepared in distilled water and the salts freshly weighed out. Some Pharmacology Departments use stock solutions of some salts, but prolonged storage is not recommended. Although physiological salt solutions may be kept for about 24 hours if stored in the fridge, because of the problems of microbial growth, fresh solutions are always preferable. Calcium chloride should be added last, and as a solution, to prevent precipitation of the bicarbonate. Cloudy solutions will result in non-viable tissues. The two most commonly used solutions are Tyrode and Krebs. As a general rule Tyrode may be used for experiments with non-innervated muscle whilst Krebs is used for nerve muscle preparations. All physiological salt solutions require aeration, either with an air pump, with carbogen (95% O_2/5% CO_2) or with oxygen depending on the salt solution used. The buffering capacity of Krebs, for example, is completed by the 5% CO_2 used in aeration bringing the solution pH to 7.4.

The majority of experiments in this book use mammalian tissues and the salt solution is warmed to the physiological temperature of 37°C. This may be reduced in some experiments for one of two reasons, either to reduce spontaneous movement of the preparation and therefore allow easier estimation of drug induced movement, or to reduce the tissue requirement for oxygen and thereby prolong the viability of the preparation (e.g. cardiac and nerve muscle preparations). The experiments involving amphibian tissue are carried out at room temperature.

2.3 Recording transducers

Many of the pharmacological experiments described require measurements of changes in length or tension. Isotonic recording allows the tissue to contract freely against a constant tension. Isometric recording measures increases in tension of the tissue when the length of the tissue is kept constant. Isotonic recording with smooth muscle probably represents a more physiological type of response than isometric recording, though in the living animal it is probable that all muscles contract partly isometrically and partly isotonically. Isotonic recording tends to produce steep dose-response curves, and this is desirable for bioassay by making the preparation more discriminating. The isometric recording method allows the tissue to remain at an almost constant position on its length-tension relationship, and relaxation of tension is relatively rapid.

Table 1. Physiological salt solutions (Ringer solutions) (salts in g/5 litre).

	Tyrode	De Jalons	Ringer Locke	Frog Ringer	Krebs	McEwens
NaCl	40.0	45.0	45.0	32.5	34.5	38.0
$NaHCO_3$	5.0	2.5	1.0	1.0	10.5	10.5
D Glucose	5.0	2.5	5.0	—	10.0	10.0
KH_2PO_4	—	—	—	—	0.8	—
NaH_2PO_4	0.25	—	—	—	—	0.72
KCl	1.0	2.1	2.1	0.7	1.8	2.1
$MgSO_4.7H_2O$	—	—	—	—	1.45	—
$MgCl_2$	0.5	—	—	—	—	—
Sucrose	—	—	—	—	—	22.5
$CaCl_2.2H_2O$ *or*	1.32	0.4	1.6	0.79	1.85	1.5
(ml/5 l of M solution)	(18 ml)	(2.7 ml)	(10.9 ml)	(5.4 ml)	(12.7 ml)	(10.1 ml)
Aeration	air	95% O_2/5% CO_2	O_2	air	95% O_2/5% CO_2	95% O_2/5% CO_2

Notes to Table 1

This table has been compiled from many sources including formulae used in several pharmacology departments in London. The disparity which exists between these teaching institutions is truly remarkable. The greatest differences relate to the calcium concentration; for example I have been able to find four different values for calcium chloride in De Jalons solution. Whether the differences are a reflection of transpositional errors throughout the years or are modifications made on academic grounds is not clear. It must be said however that despite the differences in formulations isolated tissue pharmacology is very much alive at all of these departments. The formulae in Table 1 is the best concensus of all my sources of information but the following modifications do appear to be in use and work equally well:

Tyrode

$MgSO_4.7H_2O$ 0.6 g instead of $MgCl_2$

$MgCl_2$ 1 g

$CaCl_2.2H_2O$ 1.25 g

De Jalons

$CaCl_2.2H_2O$, 0.2, 0.32, 0.38 g

$MgCl_2$ 0.025 g

Ringer Locke

D Glucose 10 g + $CaCl_2.2H_2O$ 1.5 g

$NaHCO_3$ 2.5 g + $CaCl_2.2H_2O$ 1.2 g

Frog Ringer

NaCl 30 g + $NaHCO_3$ 0.5 g + KCl 0.38 g + $CaCl_2.2H_2O$ 0.63 g

Glucose 10 g + NaH_2PO_4 0.5 g + KCl 0.14 g + $CaCl_2.H_2O$ 1 g

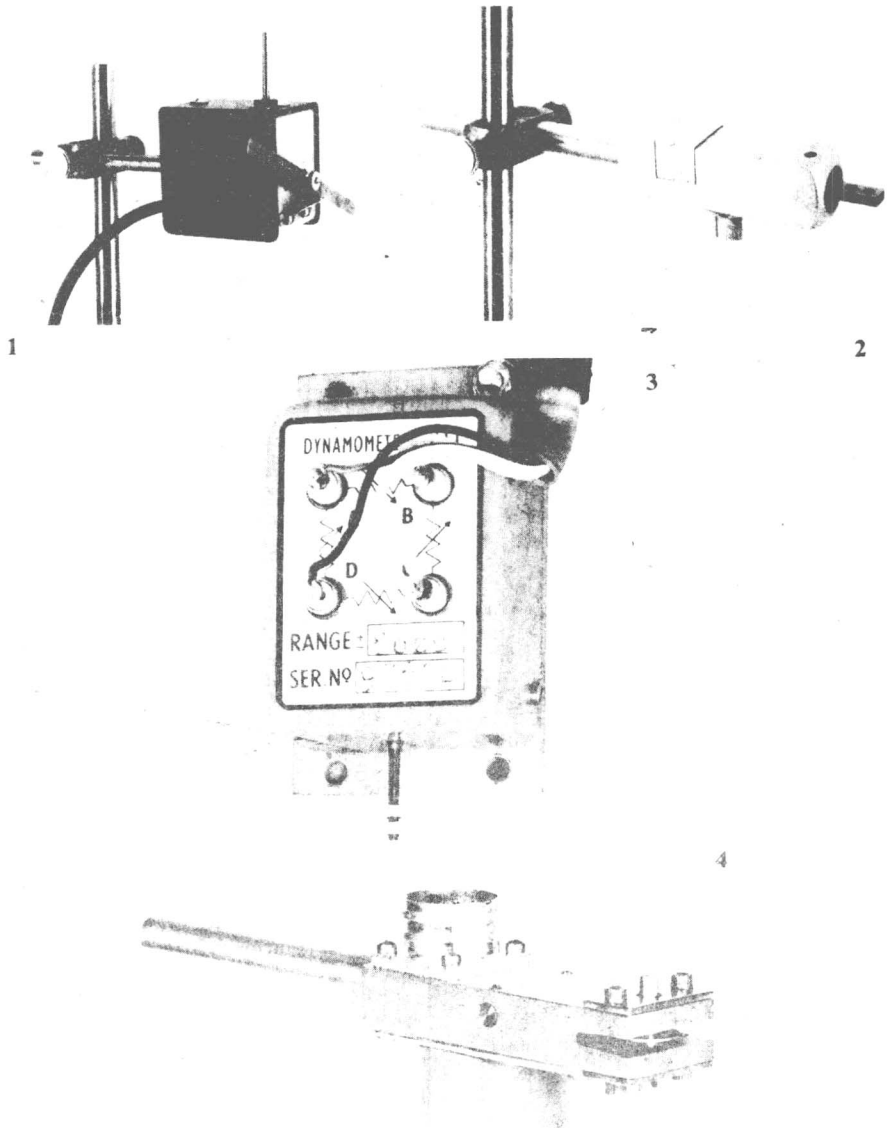

Fig. 2. Pharmacological recording transducers.
1 General purpose isotonic transducer
2 General purpose isometric transducer
3 High sensitivity isometric transducer
4 High sensitivity force displacement transducer for isometric or isotonic measurement
(Photographs provided by Harvard Apparatus (1, 2) Ormed (3) and Grass Instrument Company (4))

Relatively cheap isotonic transducers are available for measurement of length changes. Most designs have their own battery power supply and employ a lever arrangement pivoted on a fulcrum. Movement of the transducer lever is converted into an electrical potential which can be amplified and recorded as a pen movement on a chart recorder trace. Isotonic transducers can be coupled directly into flat-bed potentiometric recorders or into physiological amplifier/recorders. Isometric transducers (force displacement, or resistive strain gauges) usually consist of a cantilever arrangement connected up to four bonded strain gauges which form a fully active Wheatstone bridge network. Force applied to the rigid cantilever mechanisms is transmitted into an output voltage from the bridge circuit. This type of transducer cannot be readily used with a flat bed recorder since it requires an activating voltage to complete the full bridge circuit. It is possible to buy power supplies which serve this purpose, but some pharmacological applications of isometric recording involve fast pen movements which potentiometric recorders are unable to cope with. The more sophisticated pre-amplifier/recorder machines are therefore more desirable for use with isometric transducers. Where pre-amplifier circuitry is not required (e.g. when measuring movements which generate large tensions) bridge coupling units can be substituted, and these are substantially cheaper.

One of the disadvantages of resistive strain gauges is their sensitivity to temperature, and temperature changes will add to the change in resistance induced by mechanical activity. Generally, strain gauges are made of materials whose resistances are highly sensitive to change in dimensions and less sensitive to temperature fluctuations. It is nevertheless important to connect transducers to recording apparatus well in advance of the proposed experiment to ensure temperature equilibration..

2.4 Resting tension and equilibration

Incorrect loading of the tissue is a common error which many students commit. The resting load places the tissue under tension, and it varies for different preparations. Those which are robust and produce powerful contractions in response to drugs are given higher resting tension loads than those which only exhibit small drug-induced tension changes. It is most important that tissues are set up at the recommended resting tension; too much and the tissue will not contract in response to drugs, too little and the resting baseline will be erratic. For isometric recording the bridge of the strain gauge is balanced to the pre-set zero baseline with the resting tension loading weight attached to the transducer. The weight is then removed and replaced

by the cotton attached to the tissue. The tension is increased using an adjustable transducer stand until the recorder pen again reaches the pre-set baseline, thus loading the tissue to the weight originally hung from the transducer. For isotonic transducers, the weight is attached at a point equidistant from the fulcrum and the point of attachment of the tissue. The lever mechanism is then balanced to the horizontal position when the tissue is secured.

The placement of resting tension baseline depends on the response being studied. For those tissues which show contraction only, the baseline is set about 0.5 cm above the lowest point of pen movement. For those tissues which exhibit relaxation only, the baseline is set about 0.5 cm below the maximum upper point of pen movement. The baseline for tissues which respond with contractions and relaxations (e.g. cardiac preparations) should be mid-trace. It is important to ensure that the recorder pens are always kept between the limits of their excursions.

Having been removed from its physiological environment, manipulated in room temperature Ringer and transferred to an organ bath it is only to be expected that preparations require some time before they will respond consistently to drugs. An effort should be made to leave tissues for the full equilibration time, and it is always a golden rule to set up the preparation before making up drugs—a rule which often is not heeded.

2.5 Dose cycle and contact time

The need for regular and consistent methodology in experiments with biological tissues cannot be too highly emphasised. A consistent resting tension, thorough washing and an undisturbed equilibration period should be followed by an experiment which sticks as rigidly as possible to a regular dosing schedule. Biological variation will always mean as much as $\pm 10\%$ in the tissue response. This figure will only be increased if the tissue is treated in an irregular fashion.

The term dose cycle refers to the time between drug additions. Time clocks can be zeroed when the drug is washed out of the bath or when running a baseline. For many preparations the recording of movement is not required continuously. Recorder pen writers should be switched off during dose cycles, but it must be remembered to run a baseline before the next drug addition. It is always tempting to reduce dose cycle times and with very rigid dosing schedules this is possible with some preparations (e.g. guinea-pig ileum). With others, however, (e.g. vas deferens) a reduction in dose cycle often leads to marked tachyphylaxis, i.e. decreased responses to drugs upon repeated dosing.

此为试读,需要完整PDF请访问: www.ertongbook.com