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The inhibitory action of zinc sulphate on the contractile activity of guinea-pig ileum

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Abstract—The present study examines the inhibitory action of zinc sulphate ($ZnSO_4$) on the contractile response of various agonists on guinea-pig isolated ileum. Different doses of agonists were selected to produce similar contractile activity, in order to compare the degree of inhibition produced by $ZnSO_4$. Preincubation of ileum with $ZnSO_4$ 1×10^{-3} or 3×10^{-3} M for 10 min dose-dependently and significantly prevented the contraction induced by acetylcholine ($1{\cdot}7 \times 10^{-8}$ M), 5-HT ($2{\cdot}4 \times 10^{-6}$ M), histamine ($5{\cdot}4 \times 10^{-7}$ M) and nicotine ($1{\cdot}7 \times 10^{-6}$ M) but not by prostaglandin E_2 (PGE_2, $8{\cdot}5 \times 10^{-9}$ M). The same doses of $ZnSO_4$ reduced the twitch contraction produced by electrical field stimulation. These findings indicate that the contractile activity of PGE_2 is mediated by a mechanism different from that of other agonists and of electrical field stimulation. It is likely that the contractile activity of PGE_2 is acting through the receptors on the ileal muscle which are not blocked by $ZnSO_4$ pretreatment.

The biological activity of the zinc ion is known for its stabilizing activity on biological membranes, especially those on mast cells (Kazimierczak & Maslinski 1974) and lysosomes (Chvapil 1973; Cho et al 1980; Pfeiffer et al 1980). Those actions are reported to be responsible for the prevention of gastric ulceration (Cho et al 1980; Pfeiffer et al 1980), heart injury (Chvapil & Owen 1977) and liver necrosis (Chvapil 1973). Similar membrane stabilizing activity has been observed with steroids (Seeman 1966) and with some of the non-steroidal anti-inflammatory drugs (Tanaka & Iizuka 1968; Mizushima et al 1970) and has been reported to be responsible for the inhibitory effect of zinc on ileal contraction (Famaey et al 1977, 1979). The purpose of our study is to correlate the stabilizing effects of zinc sulphate ($ZnSO_4$) with its inhibitory action on various contractile agonists.

Materials and methods

Male guinea-pigs, 350–400 g, were stunned and bled. Segments of ileum were used after discarding the 10 cm nearest to the ileo-caecal junction. After washing out the ileal content with Tyrode solution (composition mM: NaCl 135, KCl 5·0, $CaCl_2$ 1·8, $MgCl_2$ 1·0, Na_2HPO_4 1·0, $NaHCO_3$ 15·0, glucose 11·2), ileal segments (3–4 cm) were preincubated for 30–45 min in an organ bath containing Tyrode's solution maintained at 37°C, and aerated with 95% O_2-5% CO_2. Contractions were measured isometrically by a force displacement transducer (Grass model FT03) and displayed on polygraphs (Grass model 7P1 A). Segments were placed under an initial tension of 1·0 g.

Antagonist effects of $ZnSO_4$ (1×10^{-3} and 3×10^{-3} M) were assessed on various contractile agonists (histamine $5{\cdot}4 \times 10^{-7}$, 5-HT $2{\cdot}4 \times 10^{-6}$, acetylcholine $1{\cdot}7 \times 10^{-8}$, nicotine $1{\cdot}7 \times 10^{-6}$ and PGE_2 $8{\cdot}5 \times 10^{-9}$ M). After three reproducible responses to the agonists had been obtained (responses were submaximal and on the steep part of the dose response curve), $ZnSO_4$ was added and incubated for 10 min. The height of the contraction induced by the same concentration of agonists was again measured 5 min after washout of $ZnSO_4$. All contractile forces were expressed in g.

For electrical field stimulation experiments, intact ilea were preincubated for 30–45 min in Krebs solution (composition mM: NaCl 118·3, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, glucose 11·6). Contractions of the preparation were detected isometrically by means of a force transducer (Grass model FT03) with a resting tension adjusted to 1·0 g and displayed on polygraphs (Grass model 7P1 A). Stimulation was carried out by means of platinum electrodes, placed at the top and the bottom of the organ bath. The parameters of stimulation were rectangular pulses of 1·5 ms duration, supramaximal voltage (50–80) and a frequency of 0·1 Hz. The effects of cumulative doses of $ZnSO_4$ (3×10^{-4}, 1×10^{-3} and 3×10^{-3} M) by a stepwise increase in concentration, were assessed on the twitch responses elicited by electrical stimulation.

Acetylcholine hydrochloride (Sigma), histamine dihydrochloride (Aldrich), 5-hydroxytryptamine creatinine sulphate (Sigma), nicotine salicylate (K & K Lab.) were dissolved in distilled water. A stock solution of PGE_2 (Sigma) was made in ethanol (10 mg mL^{-1}) and diluted with 0·1 M phosphate buffer. The data were analysed for significance of differences by means of a paired 2-tailed Student's *t*-test.

Results

$ZnSO_4$ preincubation at the concentration of 3×10^{-3} M reduced the ileal contraction produced by 5-HT, histamine and nicotine by at least 90%. The inhibitory action on acetylcholine contraction was less (78·7%) when compared with these agonists (Table 1); the duration of this antagonized action persisted for more than 1 h after the washout of $ZnSO_4$ after which there was a gradual recovery of the tissue to its precontracted state. However, the contractile activity of PGE_2 was not significantly reduced by the same doses of $ZnSO_4$. The magnitude of reduction was only 22·2% at the higher dose of $ZnSO_4$ (3×10^{-3} M).

$ZnSO_4$ given at the cumulative doses of 3×10^{-4} or 1×10^{-3} M did not significantly affect the supramaximal voltage-induced ileal contraction ($n = 6$, $108 \pm 6\%$ and $100 \pm 4\%$ of the control, respectively). However, at the dose of 3×10^{-3} M, ZnSO4 markedly reduced the contraction by $82 \pm 6\%$ (Fig. 1).

Discussion

$ZnSO_4$ markedly reduced the contractile force of guinea-pig ileum induced by acetylcholine, histamine (direct agonist), 5-HT (partial indirect agonist), nicotine, and electrical field stimulation (indirect stimuli). However, the ileal responses to PGE_2 were only partially prevented (less than 22%) by the same doses of $ZnSO_4$. The differential effects of $ZnSO_4$ on PGE_2 and on other contractile agonists suggest that the contractile mechanism of PGE_2 is quite different from those of acetylcholine, histamine, 5-HT, nicotine and electrical field stimulation. Furthermore it is likely that the contraction of ileum produced by electrical stimulation is mediated by endogenous release of acetylcholine and possibly other contractile mediators other than PGE_2. This finding could also explain the inhibitory action of $ZnSO_4$ on electrical vagal stimulation-induced gastric contraction in-vivo (Cho & Ogle 1977) and it is likely to be acetylcholine-mediated.

Prostaglandins are known to play a role in the contraction of intestinal longitudinal smooth muscle. The activity was found to be most potent among the agonists tested in the present study.

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Table 1. Effects of $ZnSO_4$ preincubation on the contractile activity of agonists.

Contractile agonists (M)	$ZnSO_4$ (M)	Contractile force in g		% inhibition on contractile force by Zn	P
		Before Zn	After Zn		
Acetylcholine	1×10^{-3}	3·09 ± 0·18	2·60 ± 0·38	16	> 0·1
$1·7 \times 10^{-8}$	3×10^{-3}	2·72 ± 0·44	0·58 ± 0·23	79	< 0·005
5-HT	1×10^{-3}	2·99 ± 0·23	1·92 ± 0·55	36	> 0·1
$2·4 \times 10^{-6}$	3×10^{-3}	3·24 ± 0·28	0·32 ± 0·21	90	< 0·001
Histamine	1×10^{-3}	3·50 ± 0·23	2·36 ± 0·52	33	> 0·05
$5·4 \times 10^{-7}$	3×10^{-3}	3·76 ± 0·14	0·16 ± 0·10	96	< 0·001
Nicotine	1×10^{-3}	3·25 ± 0·07	3·12 ± 0·12	4	> 0·1
$1·7 \times 10^{-6}$	3×10^{-3}	3·23 ± 0·19	0·10 ± 0·10	97	< 0·001
PGE_2	1×10^{-3}	2·84 ± 0·19	2·41 ± 0·33	15	> 0·1
$8·5 \times 10^{-9}$	3×10^{-3}	3·42 ± 0·32	2·66 ± 0·16	22	> 0·1

Data shown are means ± s.e.m. of 5 to 6 samples from at least 5 separate animals. P values indicate significantly different from the corresponding contractile force before Zn was added.

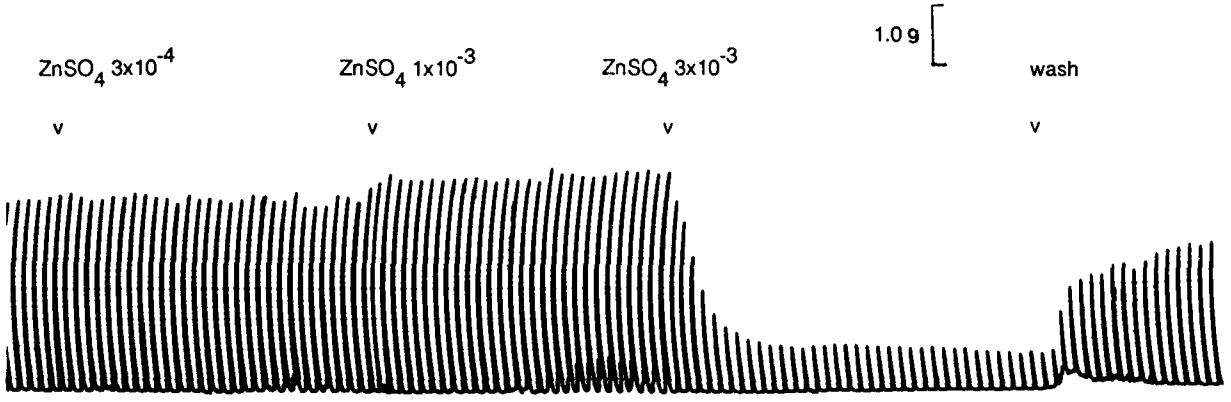

FIG. 1. The cumulative dose-response of $ZnSO_4$ (3×10^{-4}, 1×10^{-3} and 3×10^{-3} M) on the twitch contraction induced by supramaximal voltage stimulation (60 V, 0·1 Hz and 1·5 ms).

Although the action has been postulated to be due to the sensitization of other stimuli (Famaey et al 1977), increased acetylcholine release from the myenteric plexus (Schulz & Cartwright 1976) and direct action on smooth muscle cells (Bennett et al 1975), the present experimental data suggest that the contractile response of PGE_2 seems to be contributed largely by the direct action on the smooth muscle, through PGE_2 receptors and this action cannot be blocked by $ZnSO_4$. Zinc chloride has been reported to prevent lipid peroxidation of the cell membrane (Chvapil 1973) which could account for the decrease of prostaglandin synthesis and release (Cho & Pfeiffer 1982); however, the action of PGE_2, once it is released, cannot be modified by $ZnSO_4$ even at higher doses.

The mechanism by which $ZnSO_4$ prevents the contractile response of histamine, 5-HT, nicotine and acetylcholine, is still unclear. However, this antagonising action can partially explain the preventive action of $ZnSO_4$ on various kinds of gastrointestinal ulcers which are produced by excessive release of these endogenous substances from the damaged organs (Cho 1989).

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Book Review

Physiological Pharmaceutics. Biological Barriers to Drug Absorption

Clive G. Wilson and Neena Washington
Published 1989 Ellis Horwood, Chichester
186 pages
ISBN 0 7458 0543 4 £35.00

As suggested by the title, this book tackles the problem of effective delivery of pharmaceuticals by considering the properties of biological barriers that drugs will encounter when administered to the patient. The book begins with a short overview of features common to all such barriers as a good reference point for the chapters that follow. As the most often used mode of administration for drugs is still the oral route, it is not surprising that over half of the text is devoted to barriers encountered in the 'tube that links the mouth to the anus'—to borrow a phrase from the authors' preface—with a logical sequence of five chapters on the oral cavity, the oesophagus, the stomach, the small intestine and the large intestine. Non-enteral barriers are covered in the final four chapters on the skin, the eye, the nose and the lung.

Each of the nine main chapters begins with a review of the anatomy and physiology of the specific organ, within the context of its normal function. These sections are clearly written and illustrated with standard text-book type diagrams and are followed by discussions of the features more specifically related to the problems of drug absorption. Here the text relies for its examples on the recent research literature, much of it from the laboratories of the authors and their associates.

I found this an excellent book. It can serve as a reliable reference book to the physiology and anatomy of the barriers described, both for the student and for the research worker who may not be a physiologist. The subject matter is sensibly organised into logically separated chapters with each chapter having its own flow unconstrained by artificial sub-headings. Throughout the text the authors—including the guest authors for several chapters—have presented only what the reader really wants to know and avoided the tedium of any sort of catalogue. Examples from the recent literature are well-chosen to illustrate the established wisdom in the field. Contentious research is not ignored, but gently put in perspective. The result is a firm and reliable base for any researcher beginning to enquire into the ways of overcoming, or using, biological barriers to drug absorption.

A mathematical treatment of the absorption through the barriers is not pursued, rightly I believe, in a book of this type. However, there are enough examples of quantitative experiments to allow the reader to appreciate the scale of the phenomena being described; this is not always so in physiological text-books.

The authors, in their preface, excuse the omission of other routes of administration such as subcutaneous, intravenous and vaginal, because of restraints of time and length of the text; this does not detract from the present volume as the thorough treatment given here is preferable to a shallow overview that could result in a wider coverage. Nevertheless, they do state their intention of including these routes in a further text, 'providing they can raise the energy and courage necessary'. A further volume to this high standard would be a welcome addition to the series.

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