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Spasmolytic effects of cadmium and zinc ions upon the guinea-pig isolated ileum preparation

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Summary

1. An investigation has been made of the effects of cadmium and zinc ions upon the contractile response of the guinea-pig isolated ileum to methacholine, histamine, potassium ion and a.c. field stimulation. The metal ions depress the response to all of these agents.

2. Radioisotope studies showed that cadmium and zinc ions have very much larger apparent volumes of distribution than sorbitol, both within whole ileum and within strips of the longitudinal smooth muscle layer. The results of these studies were indicative of surface binding and/or intracellular accumulation of these ions.

3. It is suggested that cadmium and zinc ions directly depress smooth muscle contractility in a non-specific manner. This action may result from their binding to or accumulation within the muscle cells.

Introduction

There have been several reports (Ambache, 1946 ; Eichler & Lippert, 1966) of the ability of trace metal ions to cause spasm of the guinea-pig isolated ileum preparation. Schnieden & Weston (1969) showed that manganese (in concentrations of 10^{-6} to $6{\cdot}4 \times 10^{-5}$M) is a spasmogen whose action is mediated via the release of acetylcholine from intramural nerves. In higher concentrations manganese has spasmolytic effects (Weston, 1968).

These observations prompted an investigation of the effects of cadmium and zinc ions upon the ileum. Preliminary experiments showed that over the concentration range 10^{-7} to 10^{-4}M these ions were devoid of spasmogenic effects but were able to reduce the response of the ileum to a standard dose (50 ng/ml) of methacholine.

Hence it was postulated that these ions either do not share the ability of manganese to release sufficient acetylcholine to cause a contraction or that any such action was masked by a more potent and direct depression of smooth muscle contractility. This paper presents the results of experiments designed to test the hypothesis that cadmium and zinc ions have a direct spasmolytic effect on smooth muscle cells and to measure the volume of distribution of these ions within the tissue.

Methods

The guinea-pig ileum was set up as described by Schnieden & Weston (1969) except that the tissue was bathed with a physiological salt solution (see below)

bubbled with 100% oxygen. Contractions of the ileum were recorded using an isotonic frontal writing lever and a smoked drum. The recording system magnified changes in the length of the tissue by a factor of seven. The load imposed upon the tissue was 1 g. Where applicable, test and control experiments were run simultaneously using adjacent pieces of ileum.

Dose cycles

For histamine and methacholine a 3 min dose cycle was adopted. Drug contact time was 20 s and the physiological salt solution was changed at 20 s and at 2 minutes. Eight-point log concentration/effect curves were constructed for both these agents using doubling concentrations. The concentrations used ranged from 12·5 to 1600 ng/ml for methacholine and from 50 to 6,400 ng/ml for histamine.

As soon as the initial log concentration/effect curve had been constructed, exposure of the test preparation to physiological salt solution containing a known concentration of the metal ion commenced. A 15 min interval was allowed to elapse before construction of the second log concentration/effect curve in the presence of the metal ion.

Following this a further 15 min were allowed to elapse before construction of a third curve. In this fashion a series of log concentration/effect curves was obtained in the presence of a given concentration of the metal ion. The control preparation was treated similarly except that the metal ion was omitted.

The experiments with potassium ion were performed in a similar fashion but atropine (250 ng/ml) and tetrodotoxin (10^{-7} g/ml) were present throughout. Potassium ion was administered according to a 5 min dose cycle. Tissue contact time was 10 s and the physiological salt solution was changed at 10 s, 2 min, and 3 minutes. Four-point log concentration/effect curves were constructed using doubling concentrations of potassium ion from 5×10^{-3} to 4×10^{-2}M.

Experiments with a.c. field stimulation

For the direct electrical stimulation of intestinal smooth muscle a method similar to that used by Neal (1967) was used. A mains transformer with multiple secondary tappings was used to deliver a.c. stimuli of 12 V at 50 Hz for 15 s every minute. The shocks were delivered via two platinum ring electrodes—one situated at the top and the other at the bottom of the tissue bath. The interelectrode distance was 40 mm.

All experiments with a.c. field stimulation were carried out in the presence of atropine (250 ng/ml) and tetrodotoxin (10^{-7} g/ml). Once contraction height became constant a dose of the metal ion was applied to the tissue. The metal ion was left in contact with the tissue for 10 min or until the contraction height again became constant. The tissue was then washed with fresh physiological salt solution at 5 min intervals until the contraction height returned to its initial value or showed no further signs of change.

Measurement of the accumulation of ^{14}C-labelled sorbitol by the tissue

A method similar to that described by Foster (1967) was used. Segments of ileum weighing approximately 0·1 g were cleared of luminal contents and incubated in a

10 ml bath of physiological salt solution at 37·5° C containing the labelled sorbitol in a concentration sufficient to yield an activity of 100 nCi/ml. In some experiments strips of the longitudinal smooth muscle layer of the ileum were prepared by the method of Ambache (1954). These strips were cut into segments weighing approximately 0·05 g and were treated in a similar fashion to the segments of whole ileum.

Incubation times of 2, 4, 8, 16, 32 and 64 min were used to follow the accumulation of sorbitol in adjacent pieces of tissue. At the end of its incubation period each piece of tissue was removed from the bath, dipped briefly in fresh physiological salt solution and blotted gently on filter paper. The tissue was weighed and ground up in a mortar with a small amount of acid washed sand and 2 ml of 0·4 N perchloric acid. The extract was shaken and allowed to stand for 15 minutes. It was then centrifuged at 3,820 *g* for 10 minutes.

One millilitre of the supernatant fluid from the tissue extract (neutralized with 4 N sodium hydroxide solution) and 1 ml of the bath fluid were each subjected to a radioassay of ^{14}C by liquid scintillation counting. Quenching was assessed by the use of an internal standard. The phosphor used had the following composition: absolute methanol, 100 ml; ethylene glycol, 20 ml; naphthalene, 100 g; 2,5 diphenyloxazole, 7 g; 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl) benzene, 0·3 g; *p*-dioxane to 1000 ml.

Measurement of the accumulation of ^{115m}cadmium by the tissue

The method was similar to that used for the ^{14}C-labelled sorbitol. The concentration of cadmium in the bath fluid was $0{\cdot}35 \times 10^{-6}$M and the activity of ^{115m}cadmium was 130 nCi/ml. The ^{115m}cadmium content of the tissue extract and of the bath fluid samples were measured by liquid scintillation counting.

Measurement of the accumulation of ^{65}zinc by the tissue

The method was very similar to that used for the ^{14}C-labelled sorbitol. The concentration of zinc in the bath fluid was $0{\cdot}94 \times 10^{-6}$M and the activity of ^{65}zinc was 250 nCi/ml. The tissue extract and bath fluid samples were radioassayed for ^{65}zinc in terms of its γ-emission using a well-type sodium iodide crystal scintillation counter.

Drugs and solutions

In order to prevent the metal ions from precipitating as the respective carbonate or phosphate when added to the bathing fluid a special physiological salt solution was formulated, with the following composition (mM): Na^+, 143; K^+, 5·93; Ca^{++}, 2·55; Mg^{++}, 1·2; Cl^-, 155; SO_4^{--}, 1·22; dextrose, 11·1; 2-amino-2-(hydroxymethyl)-propane-1: 3-diol, (Tris), 2·0. The physiological salt solution was gassed with 100% oxygen and its pH at 37·5° C was 7·4. Preliminary experiments showed that this bathing fluid yielded tissue responses to methacholine which were not significantly different to those obtained in Krebs solution either in terms of the E.D. 50, maximal response, or slope of the log concentration effect curve. Similar results were obtained for the other agonists used in the present study (Small, 1968).

The drugs used were atropine sulphate, cadmium chloride, ^{115m}cadmium chloride, histamine acid phosphate, methacholine chloride, potassium chloride, tetrodotoxin citrate, zinc chloride and ^{65}zinc chloride. All drug concentrations are expressed in terms of the weight of the base per ml except for the metal ions which are expressed in terms of molarity.

Statistical methods

A two-tailed Wilcoxon matched pairs signed ranks test was used to measure the probability that differences between mean responses in related log concentration/effect curves arose by chance. In the radioisotope studies a two-tailed Mann-Whitney U test was used to measure the probability that differences between the mean volumes of distribution of the metal ions and that of sorbitol arose by chance.

Results

Experiments with methacholine, histamine and potassium ions

Control experiments showed that the log concentration/effect curves to all three agents remained constant in shape and position when interposed with 15 min intervals.

Cadmium ion in concentrations from 10^{-5} to 8×10^{-5}M depressed the response of the ileum to all three agents. The antagonism was insurmountable and the depression of the maximal response increased both with an increase in the concentration of the metal ion and with an increase in exposure time. The effects of cadmium ion on the responses of the tissue to methacholine, histamine and potassium ion are shown in Figs. 1, 3 and 5 respectively.

Similar effects were observed with zinc ion in concentrations from 10^{-5} to $1{\cdot}6 \times 10^{-4}$M, although at a dose concentration of 10^{-5} there was initially a small non-significant leftward shift of the log concentration/effect curves to methacholine and histamine. However, this developed into an insurmountable antagonism with continued exposure of the tissue to the metal ion.

The effects of zinc ions upon the responses of the tissue to methacholine, histamine and potassium ion are shown in Figs. 2, 4 and 5 respectively.

Experiments with a.c. field stimulation

In the absence of the metal ions contraction height to a.c. field stimulation remained quite stable. Cadmium ion in concentrations of 10^{-7} to 10^{-6} caused no significant change in contraction height even after a 10 min contact time. Concentrations of cadmium ion of $4{\cdot}7 \times 10^{-6}$, 10^{-5} and 10^{-4}M all produced a significant reduction in contraction height after a contact time of 1 minute. The rate of depression of contractility increased with increasing metal ion concentration.

Zinc (10^{-7}M) caused a slight but significant increase in contraction height after 10 min contact with the tissue. Concentrations between 10^{-7} and 10^{-4}M caused no consistent increase or decrease in contraction height. 10^{-4} and 10^{-3}M zinc both caused a significant reduction in contraction height after only 5 min exposure time.

The effects of cadmium and zinc ions on contraction height to a.c. field stimulation are shown in Fig. 6.

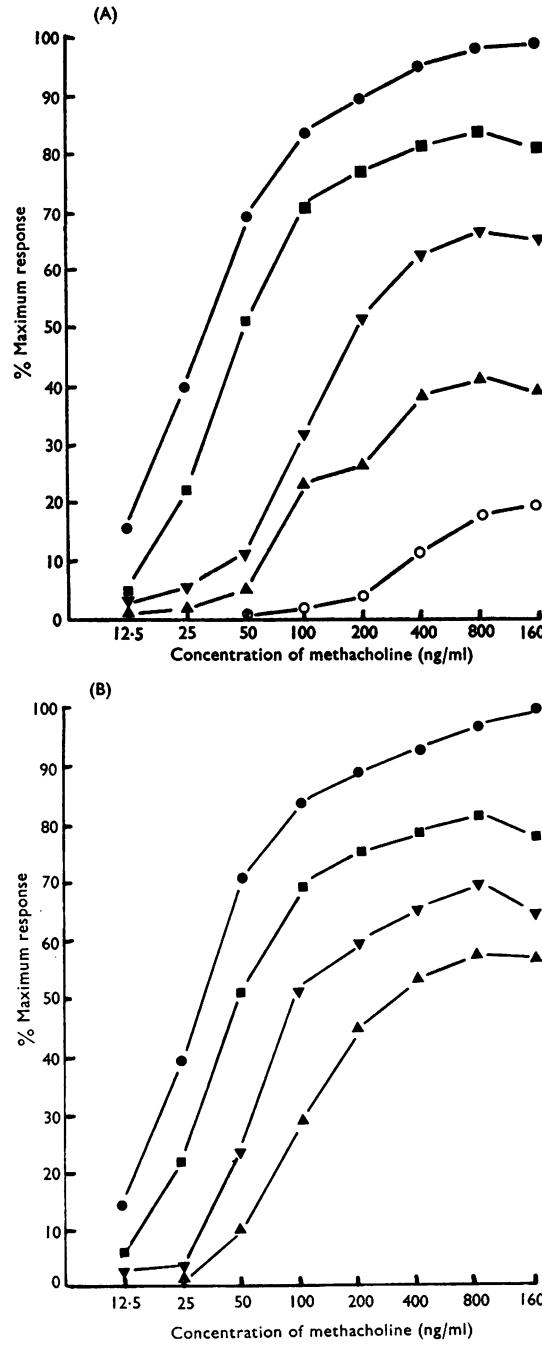

FIG. 1. Guinea-pig ileum. The effect of cadmium ions upon the response to methacholine. All points represent the means of at least six experiments. (A) The effect of concentration of cadmium ion following a tissue contact time of 15 minutes. ●——●, Controls; ■——■, 10^{-5}M cadmium ion present; ▼——▼, 2×10^{-5}M cadmium ion present; ▲——▲, 4×10^{-5}M cadmium ion present; ○——○, 8×10^{-5}M cadmium ion present. (B) The effect of tissue contact time on the action of 10^{-5}M cadmium ion. ●——●, Controls; ■——■, after 15 min exposure to cadmium; ▼——▼, after 51 min exposure to cadmium; ▲——▲, after 87 min exposure to cadmium. In both (A) and (B) all responses in the presence of the metal ion were significantly ($P<0.05$) less than the corresponding response in the control curve.

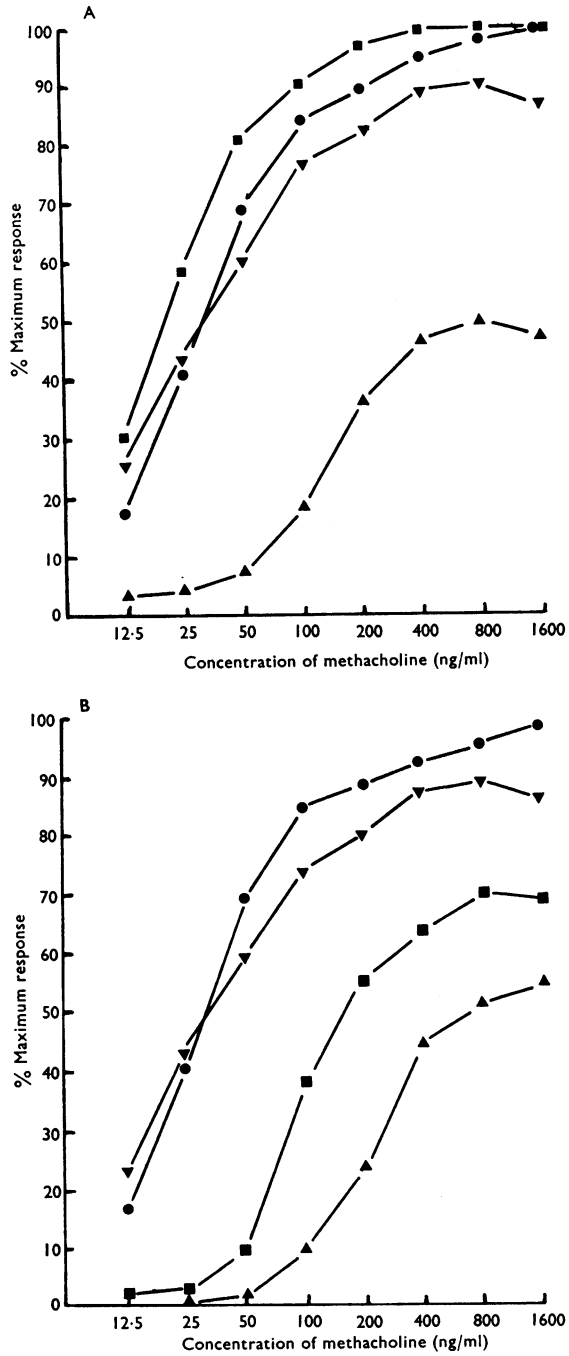

FIG. 2. Guinea-pig ileum. The effect of zinc ions upon the response to methacholine. All points represent the means of at least six experiments. (A) The effect of concentration of zinc ion following a tissue contact time of 15 minutes. ●——●, Controls; ■——■, 10^{-5}M zinc ion present; ▼——▼, 4×10^{-5}M zinc ion present; ▲——▲, $1{\cdot}6\times10^{-4}$M zinc ion present. In the case of zinc ($1{\cdot}6\times10^{-4}$M) all responses were significantly ($P<0{\cdot}05$) less than the corresponding responses of the control curve. (B) The effect of tissue contact time on the action of 4×10^{-5}M zinc ion. ●——●, Controls; ▼——▼, after 15 min exposure to zinc; ■——■, after 51 min exposure to zinc; ▲——▲, after 87 min exposure to zinc. All responses in the curves after 51 min and 87 min were significantly less than the corresponding responses of the control curve.

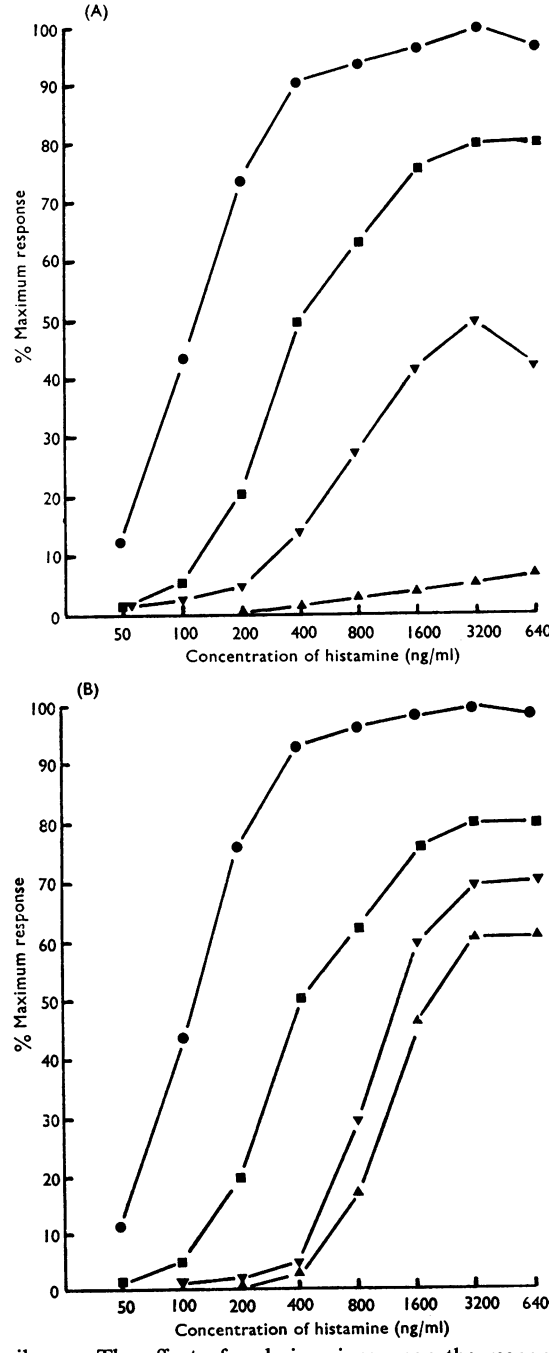

FIG. 3. Guinea-pig ileum. The effect of cadmium ions upon the response to histamine. All points represent the means of at least six experiments. (A) The effect of concentration of cadmium ion following a tissue contact time of 15 minutes. ●——●, Controls; ■——■, 10^{-5}M cadmium ion present; ▼——▼, 2×10^{-5}M cadmium ion present; ▲——▲, 4×10^{-5}M cadmium ion present. The differences between the responses of the control curve and the corresponding responses in the presence of cadmium ion were significant ($P<0.05$) at all doses of histamine. (B) The effect of tissue contact time on the action of 10^{-5}M cadmium ion. ●——●, Controls; ■——■, after 15 min exposure to cadmium; ▼——▼, after 51 min exposure to cadmium; ▲——▲, after 87 min exposure to cadmium. The differences between the responses of the control curve and the corresponding responses in the presence of cadmium ion were significant ($P<0.05$) at all doses of histamine.

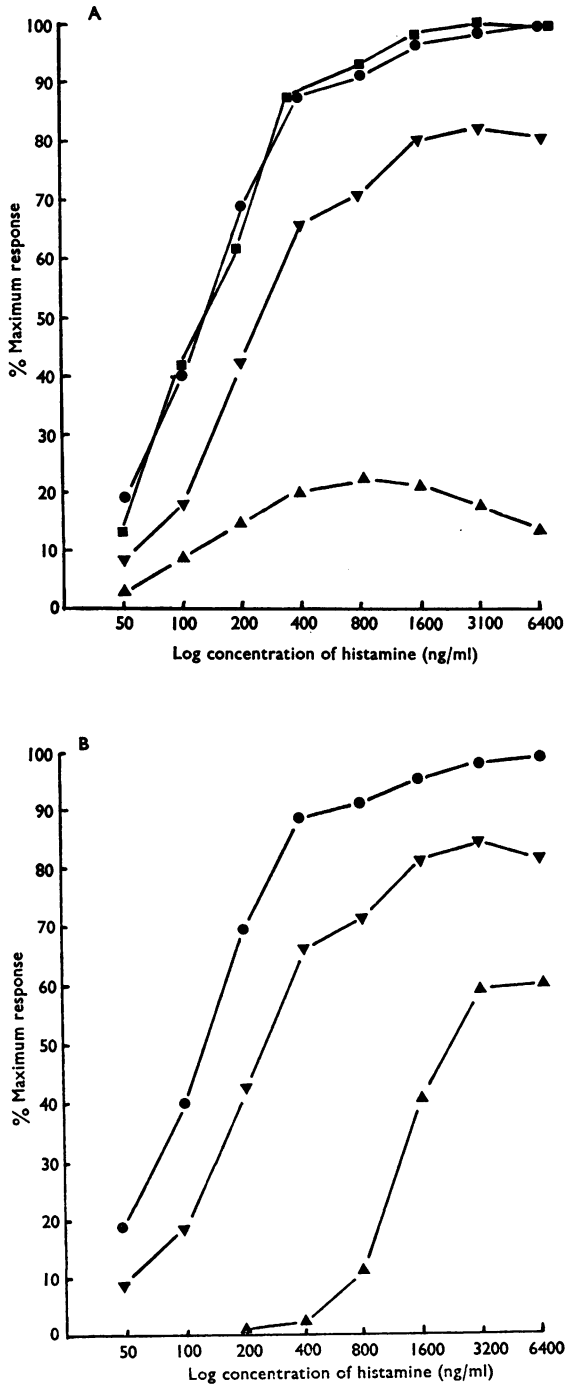

FIG. 4. Guinea-pig ileum. The effects of zinc ions upon the response to histamine. All points represent the means of at least six experiments. (A) The effect of concentration of zinc ions following a contact time of 15 minutes. ●——●, Controls; ■——■, 10^{-5}M zinc ion present; ▼——▼, 4×10^{-5}M zinc ion present; ▲——▲, $1{\cdot}6\times10^{-4}$M zinc ion present. (B) The effect of tissue contact time on the action of 4×10^{-5}M zinc ion. ●——●, Controls; ▼——▼, after 15 min exposure to zinc; ▲——▲, after 51 min exposure to zinc. In both (A) and (B) all responses in the presence of 4×10^{-5}M zinc or $1{\cdot}6\times10^{-4}$M zinc were significantly ($P<0{\cdot}05$) less than the corresponding responses of the control curve.

Radioisotope studies

The values obtained for the apparent volumes of distribution of ^{14}C-labelled sorbitol, ^{115m}cadmium and ^{65}zinc in whole ileum and in strips of the longitudinal smooth muscle layer are shown in Table 1.

Discussion

There is evidence (Botting & Turner, 1966 ; Harry, 1963 ; Small, 1968), that the action of methacholine upon the intestine of the guinea-pig is mediated largely by a direct effect upon the muscarinic receptors of the smooth muscle cells. Many workers (Emmelin & Feldberg, 1947 ; Feldberg, 1951 ; Day & Vane, 1963 ; Gershon, 1967 ; Henderson, Ariëns & Simonis 1968), have reported that histamine too, is a directly acting spasmogen.

On the basis that cadmium and zinc ions were each able to reduce the response of the tissue to both these agents it is suggested that the metal ions directly depress smooth muscle contractility in a non-specific manner. In the case of histamine, however, an alternative explanation of the inhibitory effects of the metal ions is possible. Chenoweth (1956) suggested that histamine is a potential chelating agent.

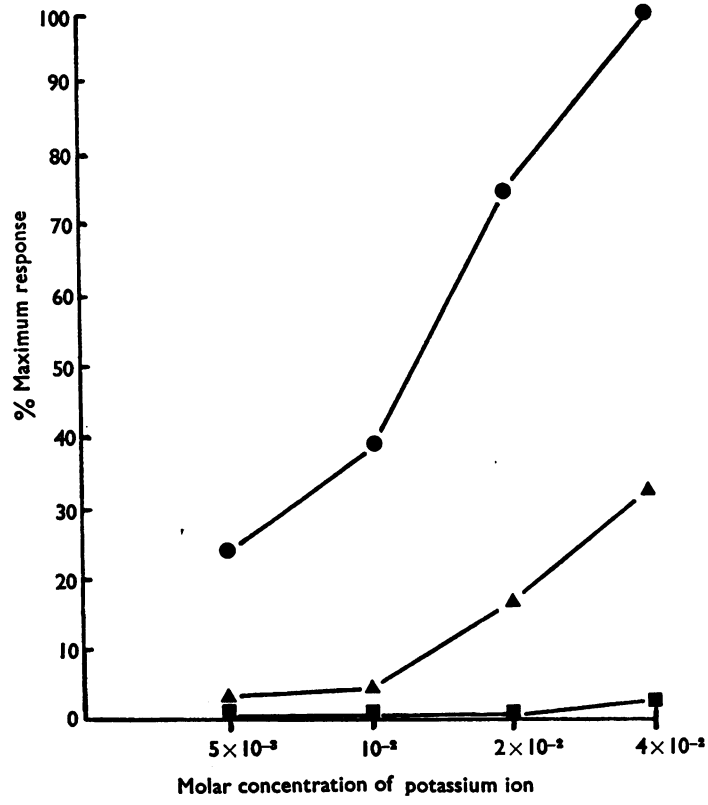

FIG. 5. Guinea-pig ileum. The effects of cadmium and zinc ions upon the atropine and tetrodotoxin resistant response to potassium ion. Each point represents the mean of six experiments. All responses in the presence of the metal ions were significantly ($P<0.05$) less than the corresponding responses of the control curve. ●—●, Control ; ■——■, after 15 min exposure to 4×10^{-5}M cadmium ion ; ▲——▲, after 15 min exposure to 4×10^{-5}M zinc ion.

If the chelates produced by interaction of histamine with cadmium or zinc are pharmacologically inert then this would afford a partial explanation of the ability of the metals to reduce the histamine response. However, the insurmountable nature of the antagonism produced by the metals rules out the possibility that chelation alone is responsible for these effects.

Gershon (1967), has analysed the tetrodotoxin resistant response of the ileum to potassium ion and has concluded that it is due to the direct stimulation of smooth

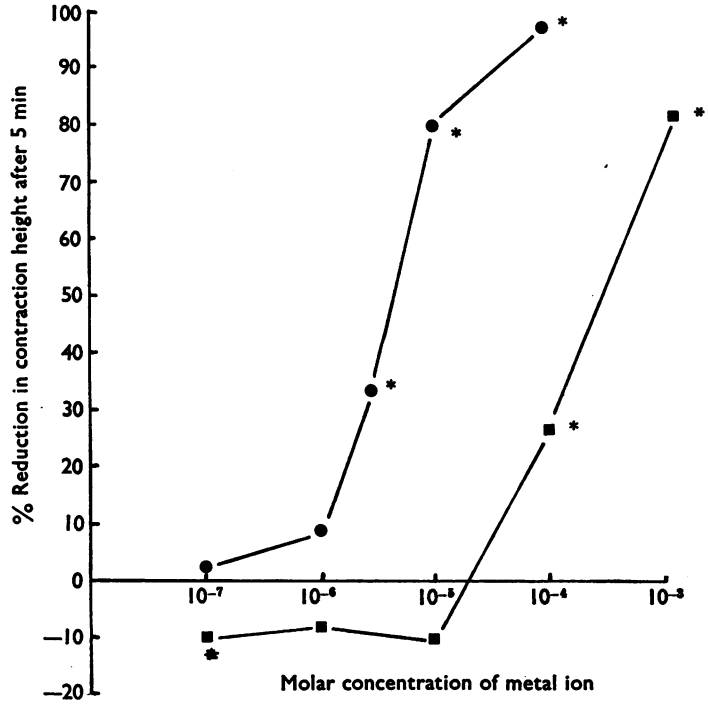

FIG. 6. Guinea-pig ileum. The effects of cadmium and zinc ions upon the atropine and tetrodotoxin resistant response to a.c. field stimulation (12 V at 50 Hz applied for 15 s every minute). Each point represents the mean of six experiments. * Represents a significant ($P<0.05$) difference from the initial contraction height. ●——●, Cadmium ion; ■——■, zinc ion.

TABLE 1. *The apparent volumes of distribution of ^{14}C-labelled sorbitol, ^{115m}cadmium ion and ^{65}zinc ion in segments of whole ileum and in strips of the longitudinal smooth muscle layer*

		Incubation time (min)					
		2	4	8	16	32	64
Apparent sorbitol space	Whole ileum	9·0	10·6	13·9	22·1	28·5	31·2
	Muscle strip	40·3	56·8	60·8	59·3	54·3	56·9
Apparent cadmium space	Whole ileum	37·2	54·8	80·6	135·1	196·0	209·3
	Muscle strip	873·0	1629·0	2008·0	3368·0	7295·0	7742·0
Apparent zinc space	Whole ileum	68·3	71·6	103·8	165·5	248·0	304·1
	Muscle strip	900·5	1501·5	2512·0	4324·0	4122·0	4326·0

All results are the means of six experiments and are expressed as ml/100 g wet weight of tissue. At time=64 min the cadmium and zinc ion spaces both for whole ileum and the longitudinal muscle a yer are significantly different from the corresponding sorbitol spaces with $P<0.001$.

muscle cells. In the present study a combination of atropine (250 ng/ml) and tetrodotoxin (10^{-7} g/ml) was used to remove neurally mediated components of the action of potassium ion. Each of these drug concentrations alone was able to abolish the effects of intramural cholinergic nerve activity where this was assessed by transmural stimulation of the ileum using square wave pulses of 60 V, width 0·3 ms applied every 12 s (Small, 1968). A similar combination of drugs was used to abolish any neural component of the effect of a.c. field stimulation.

The ability of cadmium and zinc ions to reduce the atropine and tetrodotoxin resistant response of the ileum to potassium ion and to a.c. field stimulation provides supportive evidence for these ions having a direct depressant effect on smooth muscle contractility.

The extracellular fluid space of the ileum as determined by the apparent volume of distribution of ^{14}C-labelled sorbitol after 64 min incubation was 31·2%. This value lends support to the assumption made by Brownlee & Johnson (1965) that the extracellular fluid space of the ileum is equivalent to one-third of its volume.

The somewhat larger value (56·9%) obtained after the same incubation time in the strips of the longitudinal smooth muscle layer might indicate that the cells of this layer are less densely packed than those from other parts of the wall of the ileum. A second explanation might be that the tissue has undergone some degree of stretching during its removal from the organ or that a fraction of its component cells may have ruptured. Nevertheless the preparation showed quite marked spontaneous mechanical activity whilst suspended in the incubation bath.

The apparent cadmium and zinc ion spaces were also larger in the strips of longitudinal muscle than in whole ileum. Again it is not possible to say whether this represents a selective accumulation of the metal ions in smooth muscle or an effect produced by tissue damage.

The differences between the apparent volumes of distribution of the metal ions in whole ileum and in the longitudinal smooth muscle layer and the corresponding volumes of distribution of sorbitol could be explained in any or all of three ways: (1) the extracellular fluid space available to the metal ions is much greater than that for sorbitol; (2) the metal ions are surface bound to cells or structures within the extracellular space; (3) the metal ions cross the cell membrane. The first possibility alone is insufficient to account for the very great differences observed. Hence it must be assumed that there is surface binding and/or intracellular accumulation of these ions. A technique such as autoradiography would be required to test these possibilities.

It is concluded that by binding to or accumulating within smooth muscle cells cadmium and zinc ions can depress contractility in a non-specific manner.

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