prepared using plain starch. Starch in which the surfactant was incorporated in the dry state proved to be a more effective disintegrant (disintegration time 2 min) than the starch treated with solution of surfactant (disintegration time 13–16 min).

All batches of tablets formulated with surfactant-treated starch gave higher dissolution rates than those formulated with plain starch. Both T50 and T90 values of tablets prepared using plain starch were much higher than those prepared using the treated starch. Release rates of drug from tablets prepared using plain starch were also much lower; 90% of drug was not released even after 90 min. Polysorbate 80-treated starch gave better dissolution rate profiles than SLS treated starch.

Generally, tablets containing both intra- and extragranular starch disintegrated faster and released the drug more rapidly than those containing only intragranular starch. For all the batches of tablets formulated, a direct correlation was observed between the Hardness-Friability Index (HFI) and the T90 values; the higher the HFI, greater the time required for 90% of the drug to go into solution. The method of Mendes & Brannon (1968) was used to calculate the HFI values. It was also observed that tablets containing intragranular surfactant-treated starch gave higher values of HFI and T90 than those where starch was used as both intra- and extragranular disintegrants. Also SLS-treated starch gave higher values of both HFI and T90 than those where Polysorbate 80-treated starch was used.

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Metronidazole-induced myocardial depression: chemical and pharmacological studies on the role of calcium in-vitro

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The interaction of metronidazole with calcium to form a water-soluble complex has been studied by titration with ethylenediaminetetraacetate (EDTA), direct current and pulse polarographic reduction steps of the nitro-group at pH 5 and 7, and by ultraviolet absorption. Stoichiometric calculations, X-ray powder diffraction pattern of the synthesized metronidazole-calcium complex, and molecular ion peak at *m/z* 381 in the mass spectrum of this product, showed that a 2:1 complex is formed. The interaction of metronidazole with calcium on myocardial contractile performance of guinea-pig electrically-driven isolated left atria in physiological solution was also examined. Metronidazole induced a sustained, concentration-dependent depression of the tension that was reversed by changing the bathing fluid to physiological solution, and/or by adding excess calcium ion. The drug-induced negative inotropic response was antagonized competitively by increasing calcium ion in the bath, whereas noradrenaline antagonized metronidazole-induced negative inotropic responses non-competitively. Addition of the metronidazole-calcium complex to the bath did not affect normal myocardial contractile performance. The results show that metronidazole produces a direct negative inotropic effect on isolated atrial muscles by interfering with Ca^{2+}, and by preventing Ca^{2+} function in the events leading to contractile activity of atrial muscles.

Metronidazole is commonly prescribed for the treatment of trichomoniasis, amoebiasis and anaerobic infections in doses as high as 4 g daily. The troublesome side-effects associated with heavy medication include myocardial depression (Balsara et al 1975) and Quinke's oedema (Sheveliakov 1978).

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In the heart muscle, excitation-contraction coupling is believed to depend upon a superficial membrane source of calcium ion (Ca^{2+}) (Shine et al 1971; Langer 1973). Balsara et al (1975) have indicated that metronidazole induces negative inotropic responses in isolated cardiac muscle preparations. Thus, the myocardial depressant effect could be taken to imply interference of the drug with excitation-contraction coupling processes in the heart muscle.

We have recently shown that, under certain conditions, metronidazole interacts chemically with some divalent and trivalent metal cations, such as Ca^{2+}, Zn^{2+} and Ti^{3+}, to form water-soluble complexes (Essien & Femi-Onadeko 1981). Metronidazole could, therefore, be acting as a sequestering agent. Our pharmacological studies have also shown that the drug alters calcium ion-dependent processes in experimental animals while exogenous administration of Ca^{2+} quickly reverses the cumulative neuromuscular blocking effect, probably by displacing accumulated metronidazole from Ca^{2+} – receptor binding sites at the axonal membrane. Thus, the drug would be acting in a manner essentially similar to that established for aminoglycoside antibiotics by Adams & Mathew (1974), or the reversal effect could be due to replacement of Ca^{2+} ions depleted from the myomembrane by metronidazole interference. Therefore, metronidazole could be acting by disrupting the participation of the superficial Ca^{2+} membrane pool believed to represent a source of contractile Ca^{2+} in the coupling of membrane events with mechanical activity (Goodman & Weiss 1971b).

Hitherto, no report has appeared of any chemical mechanism explaining the pharmacological effect of metronidazole on the Ca^{2+}-dependent mechanical events of isolated myocardium. We have examined the chemical and pharmacological basis of the role of calcium in metronidazole-induced myocardial depression in-vitro using guinea-pig isolated atrial muscle.

Materials and methods

Compounds and reagents. Metronidazole reference sample was a United States Pharmacopeia (USP) reference standard. Metronidazole was extracted from Flagyl (May & Baker Ltd, UK) tablets and purified. Britton-Robbinson buffers were prepared as described by Beckett et al (1974). Ethylenediaminetetraacetate disodium was a BDH analytical reagent; calcium lactate pentahydrate and calcium chloride were of analytical reagent grade (Hopkins and Williams Ltd, and BDH, UK). Toluene (Aldrich Ltd, UK), acetone, methanol and tetrahydrofuran (Fisons, UK) were of the laboratory reagent grade. (−)-Noradrenaline hydrochloride was from Sigma Laboratories Ltd, UK.

Apparatus. Ultraviolet spectra were recorded with a Varian 634 spectrophotometer. A pulse polarograph (Electrochemical System PAR model 170) was used in the sampled d.c. mode with a scan speed of 10 mV s^{-1}, at a drop time of 0·5 s; and in the differential pulse mode. A single polarographic cell was used with three electrode system, and standard calomel electrode was reference. Infrared spectra (nujol mull and KBr disc) were recorded with a Pye Unicam (SP 1100) spectrophotometer. Mass spectra were recorded on Finnigan GC-Ms model 3200 mass spectrometer at ionizing potential of 70 eV. X-ray powder diffraction patterns were recorded using a Phillips X-ray diffractiometer P.W. 1130 with a horizontal Goniometer P.W. 1380. Elemental analyses were performed by Scandinavian Microanalytical Laboratories, Herlev, Denmark.

Synthesis of calcium-metronidazole complex. The complex Ca-2($C_6H_9O_3N_3$) was prepared by stirring calcium lactate pentahydrate ($C_6H_{10}O_6.Ca.5H_2O$) with excess metronidazole ($C_6H_9O_3N_3$) in toluene and refluxing for 6 h. The toluene solution was filtered, evaporated to dryness, and the resulting solid was recrystallized from tetrahydrofuran (THF) to give white crystals, m.p. 188–190 °C (decomp). The complex was characterized as $C_{12}H_{16}O_6N_6Ca$ by elemental analysis (Found; C, 37·45%; H, 4·8%, N, 21·9%; Ca, 10·55%; Calculated: C, 37·65%; H, 4·7%, N, 21·95%, Ca, 10·5%); infrared spectrum showed N–O absorption at ν max 1390 cm^{-1}; C–H stretching at ν max 2945 cm^{-1}; mass spectrum showed highest molecular ion peak at *m/z* 381 (M + 1), corresponding to the molecular ion of a Ca.2($C_6H_8O_3N_3$) complex. The X-ray powder diffraction pattern showed a highly crystalline solid.

Complexiometric titration with EDTA. 0·01 M solution of calcium salt (20 ml) was titrated with 0·01 M solution of ethylenediaminetetraacetate (EDTA) containing small quantities of magnesium ions to facilitate sharp end-point colour change of the Eriochrome Black T by the method of Vogel (1964). 20 ml portions of calcium salt (0·01 M) containing varying quantities of metronidazole were titrated similarly. The extent of complexation of metronidazole with calcium was determined from titre differences.

Electrochemical reduction-polarography. 25 ml of separate solutions of known concentrations of calcium salt and metronidazole (8×10^{-5} M) in buffer (pH 5, 7) were polarographed by the method of Essien et al (1979). Solutions containing varying quantities of calcium and metronidazole (8×10^{-5} M) were similarly polarographed to ascertain the effect of metronidazole-calcium complexation on the polarographic characteristics of nitro-reduction of metronidazole.

Spectrophotometry. The ultraviolet absorption spectra of solutions of metronidazole (10^{-4} M) in water and 0·1 M hydrochloric acid, and of metronidazole solutions containing varying quantities of calcium were recorded using water or 0·1 M hydrochloric acid as appropriate.

Pharmacological investigation. The drugs used were dissolved in distilled water immediately before use. Noradrenaline was stabilized with an equivalent amount of sodium metabisulphite. When necessary, dilutions of stock solutions were made with fresh 0·9% w/v NaCl (saline) before use. The drug doses quoted denote final organ bath concentrations and refer to the bases.

Guinea-pig isolated electrically-driven left atria. Guinea-pigs of either sex, 300–350 g were killed by stunning and exsanguinated. The left atrium was quickly excised and suspended in a 10 ml organ bath containing modified Tris-buffered solution (composition, mmol $litre^{-1}$; NaCl, 154; KCl, 5·4; $CaCl_2$, 2·5; $MgSO_4$, 0·012; glucose 11; and Tris 6), bubbled with 95% O_2 + 5% CO_2 and maintained at 32 °C under a resting tension of 1 g. The left atrial muscle strips were field-stimulated through a pair of platinum wire electrodes (see Fabiyi & Okpako 1973), using square wave pulses of 5 ms duration at 2 Hz and supramaximal voltages (10–20 V) delivered by SRI electronic stimulators. The electrically-induced contractions of the atrial muscle preparations were measured by attaching the free end of each left atrium, to a Ugo Basile force-displacement transducer connected to a pen-recording Ugo Basile microdynamometer (model 7050). The atrial preparations were left to equilibrate for 60 min (during which time the bathing fluid was changed every 15 min) to allow contractile tensions to stabilize, before they were challenged with drugs.

The effects of noradrenaline, Ca^{2+} ($CaCl_2$), and metronidazole were examined by exogenous additions of small quantities of the drug solutions to the bathing fluid. After a maximum response to a bolus of drug solution had been obtained, the bathing solution was changed 3–5 times and then replaced with fresh physiological solution. Contractile tension was allowed to stabilize and return to control values before the addition of another drug dose.

The effects of Ca^{2+} on metronidazole-induced myocardial depression were examined by determining the maximum responsiveness of each preparation to cumulative additions of Ca^{2+} ($CaCl_2$, 5·0–20·0 mM) to the bathing fluid in the absence, and in the presence, of different concentrations of metronidazole. After obtaining a maximum response to Ca^{2+}, the muscle was washed 5–7 times, and additional time was allowed for the contractile tension to stabilize and return to control values. Responses to Ca^{2+} were determined for each preparation before, during and after exposure to metronidazole. The effects of noradrenaline on metronidazole-induced myocardial depression were also examined. Control and test contractions were measured and expressed in grams of developed tension. Contractile tension observed in the presence of metronidazole was expressed as percentage of pre-metronidazole (control) values.

Measurement of the antagonizing property of metronidazole against noradrenaline and calcium. Cumulative dose-response curves were obtained with either noradrenaline or calcium until reproducible responses were obtained. Dose-response curves to a particular agonist were then repeated 30 min after the administration of three concentrations of metronidazole to the bath. Responses were expressed as a percentage of the maximum of each agonist cumulative dose-response curve, and dose ratios between the third control curve and the curves obtained in the presence of metronidazole were calculated at 50% level. Any change in the absolute maximum response with the agonists was noted.

pA_2 values for antagonism of the positive inotropic responses to noradrenaline were calculated by the method of Arunlakshana & Schild (1959) and the slope of the plots for the agonist/antagonist pair was calculated by regression analysis. Responses to calcium were treated similarly to give 'apparent' pA_2 values (Marshall & Ojewole 1977) to obtain an indication of the relative antagonist potency of metronidazole against calcium.

Analysis of results. Results are expressed as means (± standard error of means). The significance of the differences between control and test means was determined with Student's *t*-test. Values of $P \leq 0{\cdot}05$ were taken to imply statistical significance.

Results and discussion

Titration with EDTA. Complexiometric titration consistently showed that EDTA reacted with half of the calcium ions present in an equimolar mixture of metronidazole and calcium when compared with direct titration of the same concentration of Ca^{2+} ions alone. Stoichiometric calculations showed that two moles of metronidazole complexed with one gram ion of Ca^{2+} (i.e. 2 : 1 complex). This is in agreement with the highest fragment ion *m/z* 381 (M + 1) in the mass spectrum of the synthesized complex.

Polarographic behaviour. Direct current reduction steps, half-wave potentials ($E_{\frac{1}{2}}$), and differential pulse polarographic peak potentials (Ep) for the nitro-group reduction were the same for metronidazole and metronidazole-calcium complex at the pH values investigated (i.e. pH 5; $3_{\frac{1}{2}}$, −0·41 ± 0·002 V; E_p, −0·437 ± 0·003 V; and pH 7: $E_{\frac{1}{2}}$, −0·86 ± 0·005 V; E_p, −0·91 ± 0·005 V). Thus the nitro-group in the complex is free and is probably not involved in the complexation.

Spectrophotometric behaviour. Metronidazole and its calcium complex exhibited identical absorption characteristics between 400 and 220 nm wavelengths in water, with maximum at 320 nm, and 0·1 M hydrochloric acid with maximum at 270 nm. This observation probably indicates the absorption behaviour of the imidazole system and its free nitro-group, even in the complex.

Effects of metronidazole on developed tension in guinea-pig left atria. Metronidazole (30 μM to 0·5 mM) produced a concentration-dependent negative inotropic response (Fig. 1). Maximum depression of atrial contractile tension by metronidazole was always observed 5–15 min after exogenous addition of the drug to the bathing medium. After replacement of the bathing solution with metronidazole-free physiological solution 3–5 times, the inhibitory effect of metronidazole reverted to control values within 10–20 min. Atrial muscle preparations that were exposed to relatively high concentrations of metronidazole (≥0·1 mM) for longer than 10 min always

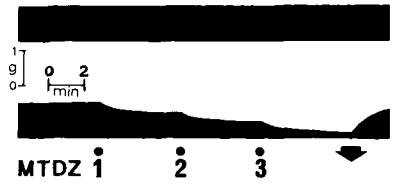

FIG. 1. Effect of metronidazole on tension developed by isolated electrically-driven left atrium of the guinea-pig. The upper trace represents saline-treated control, while the lower trace shows metronidazole-treated preparation. In the bottom trace, MTDZ (●) 1, 2 and 3 represent cumulatively-applied metronidazole, 0·125; 0·25 and 0·50 mM respectively. Metronidazole was washed off 5 times at the right hand side downward arrow.

required a longer recovery time. Absolute values of contraction (expressed as g of generated muscle tension) obtained are presented in Table 1. With each concentration of metronidazole, the decrease in contractile tension obtained after a 10 min exposure to the drug was statistically significant (see Table 1).

Table 1. Effects of cumulatively-applied metronidazole (0·05–0·50 mM) on developed tension of electrically-driven isolated atria of guinea-pigs. Each value represents the mean (±s.e.m.) of 7–9 observations.

Metronidazole (mM)	% Δ tension
0·05	−21·65 ± 3·01[a]
0·10	−32·30 ± 4·46[b]
0·20	−50·56 ± 3·87[b]
0·40	−72·53 ± 5·54[c]
0·50	−88·85 ± 5·30[c]

[a] $P < 0·05$; [b] $P < 0·01$; [c] $P < 0·001$.

Effects of calcium on metronidazole-induced left atrial depression. The effects of Ca^{2+} on myocardial depression induced by different concentrations of metronidazole (10 min exposure), as examined in 7–10 left atrial muscle preparations in normal Tris physiological solution, showed that metronidazole-provoked atrial muscle depression was markedly antagonized by Ca^{2+}. The antagonism appears competitive, since maximum Ca^{2+}-induced contractile responses were still obtainable in the presence of severe metronidazole-induced depression by increasing the Ca^{2+} ion concentration of the bathing physiological fluid.

Effects of noradrenaline on metronidazole-induced left atrial depression. Inotropic responses induced by cumulatively increasing concentrations of noradrenaline (0·5–5·0 μM) before, and after exposure of the atrial muscles to metronidazole, showed that much higher concentrations of noradrenaline (7·5–20·0 μM) were required to restore maximum control responses to noradrenaline in metronidazole-depressed atria. High concentrations of noradrenaline (7·5–20·0 μM) added to fresh preparations depressed with metronidazole without prior exposure to noradrenaline, showed that, contrary to the effect of Ca^{2+}, noradrenaline could not completely overcome the atrial depressant effect of metronidazole.

In experiments involving noradrenaline, it was consistently observed that the maximum increases in contractile tension produced in the presence of metronidazole were much less than the control maximum noradrenaline responses.

Metronidazole/agonists pA_2 determinations. The results obtained in these experiments are pA_2 noradrenaline 6·42 ± 0·45; slope 1·55; pA_2 calcium 8·44 ± 0·31, slope 1·05. Thus, metronidazole was more potent in inhibiting the positive inotropic responses induced by calcium than those produced by noradrenaline on the atrial muscle preparations. The slope of the Schild (A–S) plot for metronidazole against noradrenaline strongly suggests that metronidazole does not act as a competitive β-adrenoceptor antagonist of noradrenaline on the atrial muscle. Indeed, the relatively low pA_2 value could also be taken to imply that the antagonism between the two drugs is non-competitive.

In contrast to the noradrenaline effect, metronidazole inhibited Ca^{2+}-induced positive inotropic responses of the atrial muscles more powerfully, and its apparent potency was much greater. This is clearly evident from the relatively high 'apparent' pA_2 value obtained. The slope of the A–S plot of 1·05 could be taken to imply that the antagonism between metronidazole and calcium is competitive.

From the interaction between metronidazole and calcium, it is apparent that the two agents form a water-soluble complex which renders Ca^{2+} ions unavailable to ethylenediaminetetraacetate for complexation. This probably implicates metronidazole as a sequestering agent for calcium, even in physiological solutions.

Since isolated atria have been used to examine the effects of metronidazole on myocardial contractile performance (thereby eliminating the influence of blood flow changes or anaesthetic agents), the results presented would appear to provide unequivocal evidence for a direct negative inotropic action of the drug on myocardial contractile force.

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