Inhibition of calcium ionophore A23187-induced contractions of guinea-pig ileum by anti-inflammatory agents

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Studies on the Ca^{2+} ionophore A 23187 have shown that it releases noradrenaline from peripheral adrenergic neurons in guinea-pigs (Thoa et al 1974; Ito et al 1978), acetylcholine (ACh) from rat brain slices (Casamenti et al 1978), enzyme from rat pancreas (Heisler 1976), and histamine from rat mast cells (Foreman et al 1973; Johansen 1977; Lewis & Whittle 1977). In all these experiments, the action of the ionophore depended on the concentration of Ca^{2+} in the medium.

Non-steroid anti-inflammatory drugs (NSAIDs) inhibit the transport of Ca^{2+} across many smooth muscle membranes (Northover 1977) and it was recently shown by Lewis & Whittle (1977) that the A23187-induced histamine release from rat mast cells was also reduced by these agents. The present experiments were designed to find whether A23187-induced contractions of guinea-pig ileum were dependent on extracellular Ca^{2+} and whether the ionophore's effects could be antagonized by anti-inflammatory agents.

Pieces of proximal ileum (4·0 cm long) from male guinea-pigs were suspended in Tyrode solution (mmol $litre^{-1}$: NaCl 137, KCl 2·4, $CaCl_2$ 1·8, NaH_2PO_4 0·2, $NaHCO_3$ 11·9 and glucose 5·5). Mg^{2+} was not included because earlier experiments had shown that its absence increased the sensitivity of the tissue to A23187. Also this allowed the ionophore to be examined exclusively on calcium movements. Tissues were gassed with air and maintained at 32°C to reduce spontaneous activity. After equilibration for 30 min responses to drugs were recorded isotonically on a smoked drum (magnification 1:8).

To show the dependence of A 23187 contractions on extracellular Ca^{2+} responses to a submaximal dose of A 23187 in normal Ca^{2+} (1·8 mmol $litre^{-1}$) was repeated in the presence of reduced concentrations of Ca^{2+}. For comparison, the responses to a submaximal dose of ACh were also assessed in the same tissue along with those of the ionophore at different Ca^{2+} concentrations. The tissue was allowed to equilibrate in fresh solution for 15 min before responses to a new Ca^{2+} concentration were obtained. While the slow contractions produced by the ionophore required 6 min to give a maximum response, the contact time for ACh was 30 s. The time cycle for the ionophore was 15 min. For calculations, responses to the ionophore and ACh (6 experiments) at lower Ca^{2+} concentrations were expressed as a percent of the responses obtained with the agents in Tyrode solution with normal Ca^{2+}.

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To study the inhibitory effects of anti-inflammatory agents, a submaximal dose of A23187 selected from the dose-response curve was repeated twice and the mean response calculated. 5 min after the second dose, the inhibitory drug was added to the bath, followed 10 min later by the addition of the selected dose of ionophore. Preliminary tests showed that a contact time of 8 min was sufficient to produce the maximum inhibitory effect of the drug. The reduction in response obtained in the presence of the anti-inflammatory agent was expressed as a percent of the mean pre-drug response. After the response to A23187 had recovered, the procedure was repeated using different doses of the anti-inflammatory drug. Only one drug was tested in one tissue and at least 5 tissues were used for each drug. Statistical analysis of the results were performed using Student's *t*-test. Relative potencies of the drugs were calculated from the log dose response curves by estimating the molar concentration of each agent that inhibited the response to the ionophore by 50% (ID50 value). Stock solution (2 mg ml^{-1}) of A23187 in ethanol was kept at −15°C and freshly diluted in 0·9% NaCl (saline). NSAIDs were dissolved in 2% Na_2CO_3 and steroids in saline to obtain stock solutions of 10 mg ml^{-1}. Fresh dilutions of the stock solutions were made with saline to obtain the required molar concentrations (as the base) of each drug.

Doses employed for submaximal responses were between 50–200 ng ml^{-1} for the ionophore and 100–500 ng ml^{-1} for ACh. Responses to the ionophore decreased progressively as the Ca^{2+} concentration in the Tyrode solution was reduced from normal (1·8) to 0·9 and 0·45 mmol $litre^{-1}$ and no contractions were obtained at 0·23 mmol $litre^{-1}$. Mean (± s.e.m.) percent reduction of the responses were 50 ± 3·0 at 0·9 mmol $litre^{-1}$ and 85·1 ± 4·2 at 0·45 mmol $litre^{-1}$, these results being statistically significant at $P < 0.005$. ACh responses were less affected by the decrease in Ca^{2+} concentrations, the mean (± s.e.m.) percent reduction being 5·3 ± 1·0 at 0·9 mmol $litre^{-1}$, 20·3 ± 2·1 at 0·45 mmol $litre^{-1}$ and 45·1 ± 4·0 at 0·23 mmol $litre^{-1}$. The reductions obtained at 0·45 and 0·23 mmol $litre^{-1}$ Ca^{2+} concentrations were statistically significant at $P < 0.01$.

Results for the inhibitory actions of anti-inflammatory agents are given in Fig. 1. All the agents tested produced a dose-related inhibition of A23187-induced contractions of the ileum. The inhibitory actions of the drugs were reversible with repeated washings. The ID50 values (μ mol ml^{-1}) obtained from the graphs for NSAIDs were: meclofenamate 0·33 (0·1), indomethacin 1·0 (0·36), phenylbutazone 13·3 (4·1), aspirin 50·0 (9·0) and benoxaprofen 95·0 (29·0). The corresponding

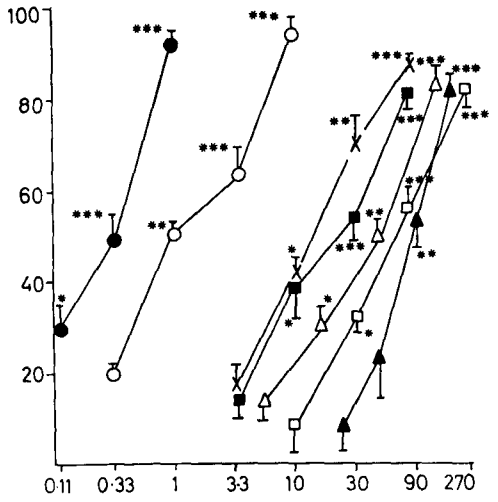

FIG. 1. Inhibitory actions of anti-inflammatory agents on contractions of guinea-pig ileum produced by ionophore A23187. Each point represents the mean ± s.e.m. of per cent reduction in ionophore-induced responses obtained from five experiments using meclofenamate (●—●), indomethacin (○—○), phenylbutazone (×—×), aspirin (△—△), benoxaprofen (▲—▲), prednisolone (■—■), or hydrocortisone (□—□). * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$. Using Student's t-test. Ordinate: Percent reduction in response. Abscissa: Log concentration (μ mol ml^{-1}) of drugs in Tyrode solution.

values for prednisolone was 22·4 (8·1) and 70·8 (25·6) for hydrocortisone. The value in brackets given with each agent represents the corresponding ED50 value in μg ml^{-1} for the individual drug. Addition to the tissue of Na_2CO_3 in concentrations present in the test solution of drugs had no effect on the ionophore-induced responses.

The findings support those of Reed & Lardy (1972) that the action of A23187 depends on Ca^{2+} concentration. The contractions produced by this agent may thus be assumed to be by increasing the free intracellular Ca^{2+} by transporting the extracellular Ca^{2+} into the cell. As shown by Takayanagi et al (1977), the ACh response, on the other hand, depends also on its ability to release Ca^{2+} from intracellular storage sites, which accounts for its lack of dependence on extracellular Ca^{2+} in the ileum.

The ID50 values for NSAIDs show that they inhibit A23187-induced increase in membrane permeability to Ca^{2+} in subtherapeutic doses and the relative potences of indomethacin, phenylbutazone and aspirin correlates with their clinical doses (Brune et al 1976). The results agree with the earlier findings by Northover (1967; 1973 and 1977) and Famey & Whitehouse (1976) who demonstrated the inhibitory actions of higher doses of NSAIDs on calcium uptake by smooth muscles and lymphoid cells respectively. Meclofenamate was the most potent, while benoxaprofen showed the least activity, even though in many anti-inflammatory tests in rats, it was more potent than phenylbutazone (Cashin et al 1977).

The larger concentrations of prednisolone and hydrocortisone required to inhibit A23187-induced contractions bear no relation to their clinical doses when compared with the NSAIDs and thus suggest that their effects on calcium transport may not be therapeutically important.

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