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Inhibition by anti-inflammatory agents of contraction induced by epidermal growth factor-urogastrone in isolated longitudinal smooth muscle strips from guinea-pig stomach

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1 Epidermal growth factor-urogastrone (EGF-URO) caused a concentration-dependent contractile response of longitudinal muscle strips from the gastric body of the guinea-pig stomach. The contractile response to EGF-URO was monophasic, with tension returning rapidly to baseline. Desensitization was evident in that further addition of EGF-URO to the organ bath did not cause a second contraction.

2 Preincubation with indomethacin, ibuprofen, naproxen and aspirin markedly inhibited the contractions induced by EGF-URO with an order of potency (indomethacin > naproxen > ibuprofen > aspirin) that reflected the ability of these agents to inhibit cyclo-oxygenase.

3 The data indicate that prostanoids mediate the action of EGF-URO in the longitudinal muscle preparation.

4 Auranofin (0.5 to 50 μM), a chrysotherapeutic agent with antiproliferative properties used for treating rheumatoid arthritis, also markedly inhibited the EGF-URO response; however, other gold-containing compounds (aurothioglucose or gold sodium thiomalate at 30 to 100 μM) failed to cause significant inhibition.

5 Preincubation of preparations for 2 h with 1 μM hydrocortisone, prednisolone or dexamethasone caused an inhibition of EGF-URO-induced contraction of approximately 50%. However, steroids lacking either a 17α-hydroxyl (corticosterone) or an 11β-hydroxyl (cortisone, deoxycorticosterone, prednisone) substituent did not inhibit the contraction caused by EGF-URO. For hydrocortisone, the inhibitory effect was half-maximal at 0.2 μM and was maximal at 1 μM. Cycloheximide (10 μM) blocked the inhibitory action of hydrocortisone and potentiated the contractile action of EGF-URO.

6 The ability of a variety of steroidal and non-steroidal anti-inflammatory agents to interfere with the action of EGF-URO in a smooth muscle preparation suggests that these agents may also inhibit the action of EGF-URO mediated by prostanoids in other target tissues.

7 The data also point to a potential role for EGF-URO in regulating gastric motility.

Introduction

Epidermal growth factor-urogastrone (EGF-URO), a polypeptide of about 6000 a.m.u., found in many mammalian species including man, has been studied extensively for its effects on cellular proliferation and on a variety of parameters associated with cell growth (Gregory *et al.*, 1977; Hollenberg, 1979; Carpenter & Cohen, 1979; Carpenter, 1981). Recently, attention has begun to focus on the ability of EGF-URO to regulate blood flow *in vivo* (Gan *et al.*, 1987) and to modulate the contractility of isolated

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arterial strips *in vitro* (Berk *et al.*, 1985; Muramatsu *et al.*, 1985; 1986; Gan *et al.*, 1987). In our own work (Muramatsu *et al.*, 1985) we have observed that the contractile effect of EGF-URO in rat isolated arterial strips can be inhibited by indomethacin, thereby implicating arachidonate metabolites as mediators of the EGF-URO response. The ability of EGF-URO to stimulate prostaglandin production has been observed in a variety of tissues including the perfused rat stomach (Chiba *et al.*, 1982), as well as in cultures of mouse fibrosarcoma (Hirata *et al.*, 1985), osteoblastic cells (Yokota *et al.*, 1986) and bone (Voelkel *et al.*, 1980). It is known that receptors for EGF-URO are present in smooth muscle-containing tissue other than the vasculature (Bharghava *et al.*, 1979; Hofmann *et al.*, 1984; Mukku & Stancel, 1985; Gardner *et al.*, 1987). Thus, we have begun to explore the action of EGF-URO in non-vascular smooth muscle preparations and to evaluate the ability of agents known to abrogate prostaglandin production to modulate EGF-URO action in such preparations. In the work we describe here, we demonstrate the ability of a variety of steroidal and non-steroidal anti-inflammatory agents to inhibit the contractile effect of EGF-URO in isolated longitudinal strips of guinea-pig stomach.

Methods

Longitudinal muscle preparation

Male guinea-pigs (350–450 g) were killed by a blow on the head, were exsanguinated from the common carotid arteries and the stomach was removed. The longitudinal muscle layer of the gastric body was carefully separated from the mucosa and muscularis mucosa under a dissecting microscope, as described previously (Miura *et al.*, 1981). The longitudinal strip was prepared by cutting at right angles against the circular muscle bundles. The width and length of the preparations were approximately 2 mm and 10 mm, respectively. Each preparation was mounted vertically in a plastic organ bath containing 3 ml of Krebs-Henseleit solution of the following composition (mM): NaCl 115, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 10; in distilled, deionized water. The bath medium was maintained at 37°C and was aerated before and during each experiment with a mixture of 95% O_2 and 5% CO_2. Under these conditions, the pH (7.4) remained constant. A resting tension of about 1.0 g was applied initially and the tissue was allowed to come to equilibrium isometrically, at which time resting tension was approximately 0.5 g. This resting tension was unchanged in the presence of nonsteroidal anti-inflammatory agents, steroids, auranofin or cycloheximide. Contractile responses were recorded isometrically through force-displacement transducers. After allowing the preparation to equilibrate for 90 min, the response to 50 mM KCl (this concentration of KCl yielded a maximal KCl response of about 0.8 g tension) was routinely recorded twice at 30 min intervals; the second contraction was used as a standard response to monitor the viability of the preparation. In some experiments done to test the ability of various agents to inhibit agonist-mediated muscle contractility, a submaximal concentration of KCl (30 mM) was used to monitor the effects of the test compounds on intrinsic muscle contractility. Drugs were added directly to the bath, and concentrations were calculated accordingly. Statistical analysis of the data was done by use of an unpaired Student's t test for two means; a value of $P < 0.05$ was considered statistically significant.

Reagents

Mouse epidermal growth factor-urogastrone was isolated from submaxillary glands of testosterone-treated male mice as previously described (Savage & Cohen, 1972). Routinely, the peptide isolated in our laboratory by this method yields a single band upon gel electrophoresis in sodium dodecyl sulphate-containing polyacrylamide gels (15%) under reducing conditions and a single peak upon reverse-phase liquid chromatography (C-19 column) using an acetonitrile gradient in 0.1% trifluoroacetic acid. In a mitogenesis assay (human skin fibroblasts) EGF-URO prepared in our laboratory and stored frozen (0.2 to 1 mg ml^{-1} in 50 mM sodium bicarbonate) has a potency (EC_{50}) of approximately 0.25 ng ml^{-1} (Hollenberg & Gregory, 1980); this value is in accord with the potency reported for murine β-EGF-URO or for murine α-EGF-URO that has been stored frozen in buffer solutions (Matrisian *et al.*, 1982). The absolute concentration of EGF-URO in stock solutions, from which appropriate dilutions were added to the organ bath, was measured spectrophotometrically. All inorganic reagents were of analytical grade. Other reagents were purchased from the following suppliers: ibuprofen, naproxen, aspirin, aurothioglucose, corticosterone, deoxycorticosterone, prednisolone, prednisone, dexamethasone, hydrocortisone, cortisone and cycloheximide, from Sigma Chemical Co.; indomethacin was from Merck Sharp & Dohme, Canada. Auranofin and gold sodium thiomalate were donated by Dr Kenneth Wong (University of Alberta, Edmonton, Alberta, Canada). Ibuprofen, indomethacin, naproxen, aspirin, auranofin and the steroids were dissolved in 100% dimethyl sulphoxide (DMSO). The other reagents were dissolved in dis-

tilled water. When present, the final concentration of DMSO in the bath was never more than 0.1% (this concentration of DMSO alone did not cause any significant effect) and was usually <0.01% v/v.

Results

Response of longitudinal muscle preparations to EGF-URO

EGF-URO caused a contraction of the preparation that began within 30–60 s, that gradually increased to a maximum at 4 to 5 min, and that returned towards the baseline tension at about 10 min (Figure 1). The response to EGF-URO showed marked desensitization, in that addition of a second cumulative dose to the organ bath failed to elicit a response (Figure 1a). If, however, the preparation was washed free from EGF-URO, which was administered at intervals of 50 min or longer, a reproducible response to a standard test concentration of EGF-URO (100 ng ml^{-1}) could be obtained (Figure 1b). In a naive preparation, the response to the first administration of EGF-URO was somewhat smaller than the reproducible response to the administration of the second and subsequent test concentrations of EGF-URO (Figure 1b). Therefore, in experiments designed to evaluate the action of the anti-inflammatory agents, the second contraction caused by a constant test concentration of EGF-URO (100 ng ml^{-1}) was used as a standard response (100%). By spacing doses at 50 min intervals, with washing the preparation between doses, it was possible to obtain a concentration-response curve for EGF-URO (Figure 2) demonstrating a minimum effective concentration of 0.3 to 1 ng ml^{-1} (0.05 to 0.17 nM), a half-maximal contraction (ED_{50}) at 3.7 ± 1.7 ng ml^{-1} (0.6 nM) (mean ± s.e.mean, for $n = 7$) and a maximal contraction at 100 to 300 ng ml^{-1} (17 to 50 nM). For the concentration-response curve, the contraction at 300 ng ml^{-1}

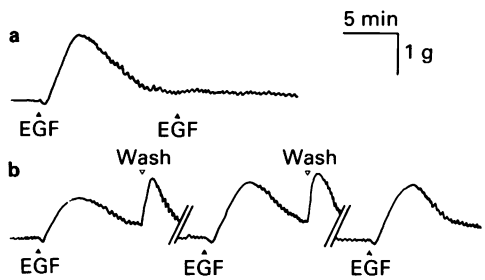

Figure 1 Epidermal growth factor-urogastrone (EGF-URO)-induced contractile response: desensitization and reproducibility. (a) Desensitization: the tracing shows a typical (one of four in a series) reproducible response to EGF-URO (▲, EGF: 100 ng ml^{-1}) added at 50 min intervals, followed by washing; the cumulative addition of EGF-URO to this preparation without washing, when tension had returned to the baseline, failed to elicit a second response. (b) Reproducibility: the tracing shows the response of a naive preparation to repeated doses of EGF-URO (▲, EGF: 100 ng ml^{-1}) added at 50 min intervals, followed at 10 min by washing (△, wash × 3). The response to the first dose of EGF-URO was slightly smaller than the reproducible response to the second and subsequent doses of EGF-URO.

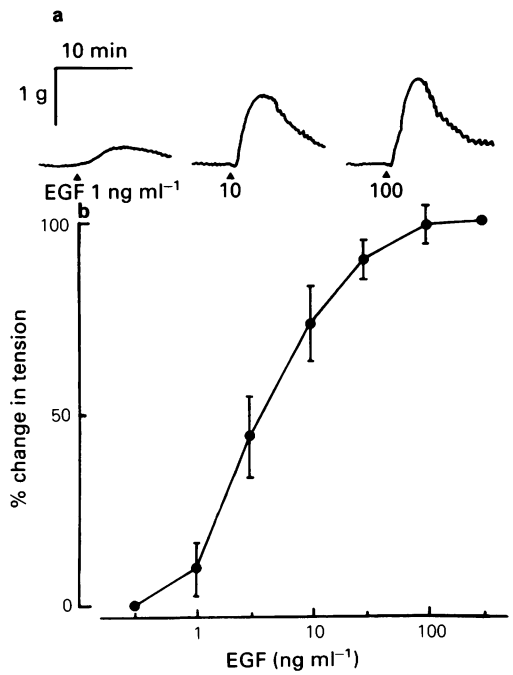

Figure 2 Concentration-response curve for epidermal growth factor-urogastrone (EGF-URO)-induced contraction. (a) Shows the typical graded responses to increasing concentrations of EGF-URO (▲, EGF 1, 10 and 100 ng ml^{-1}), from which the concentration-response curve was constructed, using the response to 300 ng ml^{-1} EGF-URO (1.4 ± 0.1 g tension, mean ± s.e.mean for $n = 7$) as 100% in each preparation. (b) EGF-URO was added at 50 min intervals and was washed from the tissue 10 min after its addition, as shown in Figure 1b. The points represent the mean values, for which the s.e.mean for 7 determinations is indicated by the vertical bars.

EGF-URO was taken as 100% (Figure 2). In an experiment where the medium (100 ng ml^{-1} EGF-URO) was withdrawn from one preparation at a time when tension was returning towards baseline (at 10 to 12 min) and was added to a second preparation, a robust response was observed that was equivalent to that of the previous test concentration (100 ng ml^{-1}) of EGF-URO (not shown). Thus, degradation of EGF-URO or the production of inhibitory compounds did not appear to be an important factor in the responsiveness of the tissue to EGF-URO.

Effects of non-steroidal anti-inflammatory agents

Preincubation of the preparation for 20 min with indomethacin, naproxen, ibuprofen or aspirin caused a concentration-dependent inhibition of the contraction induced by 100 ng ml^{-1} EGF-URO (Figure 3). In these experiments, EGF-URO was added to the bath at 50 min intervals, followed by washing, so as to obtain a reproducible contraction. The anti-inflammatory agents were added to the bath 20 min before the addition of EGF-URO (100 ng ml^{-1}) and the tissue was washed 10 min after the addition of EGF-URO. The inhibitory effects of one or two concentrations of an anti-inflammatory agent (one low concentration; one high concentration) were examined in an individual preparation with this protocol. Results from 3 to 5 independent experiments for each agent yielded the concentration-response curves shown in Figure 3. The concentrations of these agents causing a 50% inhibition of the contractile response (IC_{50}; means ± s.e.mean for $n = 3$–5) were: for indomethacin, 12 ± 8 nM; naproxen, 47 ± 12 nM; ibuprofen 150 ± 3 nM; and for aspirin, 14 ± 5 μM. These agents (at 1 μM, except for aspirin used at 10 μM) did not show any effect on the submaximal reproducible contractions caused either by prostaglandin E_2 (PGE_2) (30 nM) or by 30 mM KCl (not shown). The agents did not affect the contractile response to KCl and PGE_2 (relative to control) if added either 20 min before each agonist or if added during the sustained contraction. Nor did these agents affect the resting muscle tension.

Preincubation of the preparations for 20 min with auranofin at concentrations from 0.5 to 50 μM inhibited the EGF-URO response in a concentration-dependent manner, with an apparent IC_{50} of 4.3 ± 1.8 μM (mean ± s.e.mean for $n = 3$–6; Figure 4). In contrast, preincubation with 10 μM auranofin for 30 min had no effect on the contractile responses of the tissue to 30 nM PGE_2 or 30 mM KCl (not shown) nor did this exposure to auranofin affect resting tension. However, continued preincubation of the tissue (40 min or longer) with 10 μM auranofin caused a gradual diminution of the contractile responses caused by KCl (30 mM) or PGE_2 (30 nM) (not shown), suggestive of a cytotoxic effect of the compound, as described previously (Mirabelli & Crooke, 1983). If, after preincubation for 20 min with auranofin (10 μM), the tissue was washed before the addition of EGF-URO, the contractile response to the polypeptide was still inhibited (not shown). Thus, this action of auranofin did not appear to be spontaneously reversible and the inhibitory action did not appear to be due to a chemical reaction between EGF-URO and auranofin. In contrast with auranofin, even at comparatively high concentrations (100 μM) neither gold sodium thiomalate nor aurothioglucose caused an appreciable inhibition of the response to EGF-URO (Figure 4).

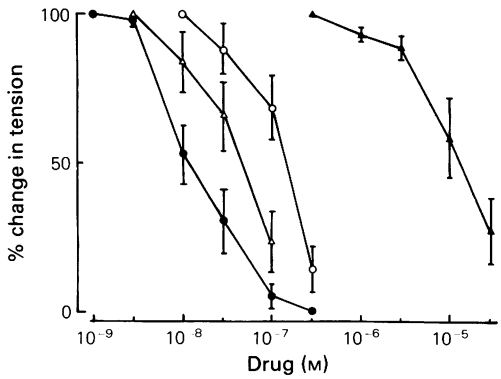

Figure 3 Dose-response curves for the inhibitory action of non-steroidal anti-inflammatory drugs on epidermal growth factor-urogastrone (EGF-URO)-mediated contraction. After obtaining a reproducible response to EGF-URO (100 ng ml^{-1}, administered for a 10 min period every 50 min, followed by washing: the second response to EGF-URO typified the reproducible contraction and was taken as 100% for the % change in tension), the tissue was exposed for 20 min at 37°C to increasing concentrations of four non-steroidal anti-inflammatory drugs: (●), indomethacin; (○), ibuprofen; (▲), aspirin; (△), naproxen. The response to a subsequent test dose (100 ng ml^{-1}) of EGF-URO, added directly to the organ bath after the 20 min preincubation period, was then measured. The % change in tension in response to the test concentration was measured relative to the standard response (1.2 ± 0.1 g, mean ± s.e.mean for $n = 17$) measured prior to drug treatment. The resting tension was taken as 0% change in each preparation. Each drug was tested at two concentrations (one low; one high) in a single tissue preparation. Results were pooled to construct the concentration-response curves. Each data point represents an average of 3 to 5 determinations, for which the vertical bars represent the s.e.mean.

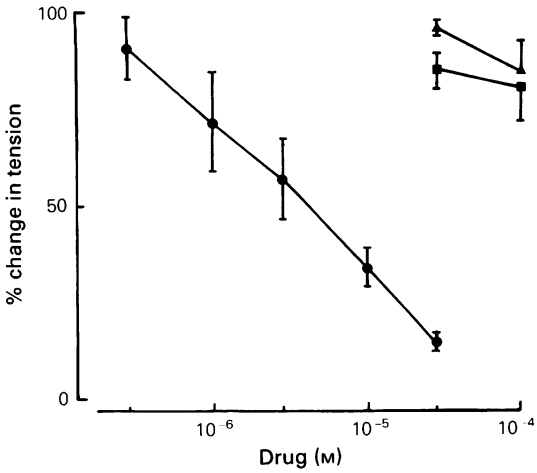

Figure 4 Effects of gold compounds on epidermal growth factor-urogastrone (EGF-URO)-induced contraction. The protocol was the same as that outlined in the legend to Figure 3. Preparations were incubated for 20 min with different concentrations of three compounds: (●) auranofin; (▲) gold sodium thiomalate; (■) aurothioglucose before the addition of EGF-URO (100 ng ml^{-1}). In this experimental series, the reproducible response (100%) to EGF-URO was 1.2 ± 0.1 g tension (mean ± s.e.mean for $n = 21$). Each data point and bar represent the mean ± s.e.mean for 3 to 6 determinations.

Effects of steroidal anti-inflammatory agents

The responses of preparations to a repeated standard test-concentration (100 ng ml^{-1}) of EGF-URO were reproducible over a 3 h period and appeared to be stable for up to 8 h. Over a 3 h period, exposure of the tissue to hydrocortisone (1 μM) caused an inhibition of the EGF-URO-induced contraction which fell to about 50% of control values 2 h after the addition of steroid (Figure 5). In contrast, neither the steroid solvent alone (0.01% v/v dimethylsulphoxide) nor the steroid analogue (cortisone, 1 μM) lacking the 11β-hydroxyl substituent required for anti-inflammatory activity had any effect on the contractile response to EGF-URO (Figure 5). Like hydrocortisone, prednisolone (1 μM) and dexamethasone (1 μM) inhibited the response to EGF-URO (Figure 6 and Table 1); in contrast, prednisone (1 μM), corticosterone (1 μM) and deoxycorticosterone (1 μM) did not show any significant inhibitory effect on the contraction caused by EGF-URO (Table 1). The concentration-response curves for the inhibitory actions of hydrocortisone, dexamethasone and prednisolone are shown in Figure 6. None of the agents was able to achieve more than a 50% inhibition. The apparent concentrations of hydrocortisone, prednisolone and dexamethasone causing half-maximal inhibition were 0.22 ± 0.18 μM, 0.22 ± 0.35 μM and 0.10 ± 0.25 μM, respectively (mean ± s.e.mean for

Table 1 Effects of preincubation with various steroids on epidermal growth factor-urogastrone (EGF-URO)-induced contraction

Agents	*Presence of 11-β and 17α-hydroxyl groups on the structure* 11β-OH	17α-OH	*Change in maximal tension* (%)	n
None			97 ± 3	4
DMSO 0.01%			100 ± 5	7
Hydrocortisone	+	+	52 ± 8***	8
Dexamethasone	+	+	57 ± 13**	5
Prednisolone	+	+	69 ± 11*	7
Cortisone	−	+	103 ± 4	5
Prednisone	−	+	109 ± 5	5
Corticosterone	+	−	93 ± 13	7
Deoxycorticosterone	−	−	98 ± 4	5

The procedure used was the same as that described in Figure 3. The second change in maximal tension induced by 100 ng ml^{-1} EGF was taken as 100% (1.2 ± 0.1 g). The data were calculated from the 4th contraction induced by 100 ng ml^{-1} EGF (i.e. 2 h preincubation with each steroid, at a concentration of 1 μM). The final concentration of dimethylsulphoxide (DMSO) resulting from the dilution of stock solutions of steroids was 0.01% v/v. *n*, number of determinations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (compared with the responses in the presence of 0.01% DMSO).

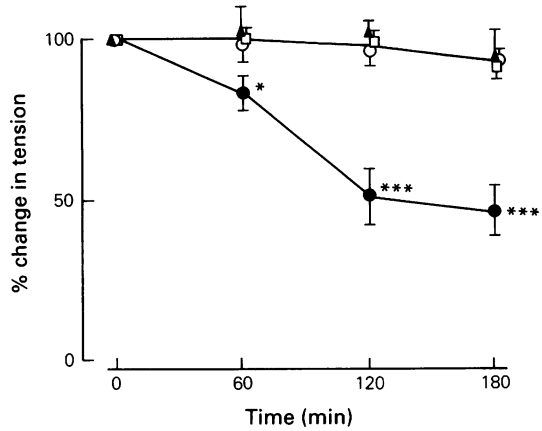

Figure 5 Time-course of the hydrocortisone-mediated inhibition of (EGF-URO)-induced contraction. The procedure was the same as that outlined in the legend to Figure 3, except that the strips were continuously exposed to steroids: (●) 1 μM hydrocortisone; (▲) 1 μM cortisone or dimethylsulphoxide (□) 0.01% v/v DMSO after the second response of the tissue to EGF-URO (100 ng ml^{-1}) had been measured (1.4 ± 0.1 g tension, mean ± s.e.mean for $n = 22$); this second standard response was taken as the 100% increase in tension. The time at which the second wash was done to free the tissue of EGF-URO was taken as $t = 0$; and the responses to subsequent doses of EGF-URO (100 ng ml^{-1}), administered for 10 min at 50 min intervals, were measured relative to the standard response. The points and bars represent the average values ± s.e.mean; the number of determinations was: control 4; DMSO 7; hydrocortisone 6; cortisone 5. * $P < 0.05$; *** $P < 0.001$ (compared with the response in the absence of hydrocortisone).

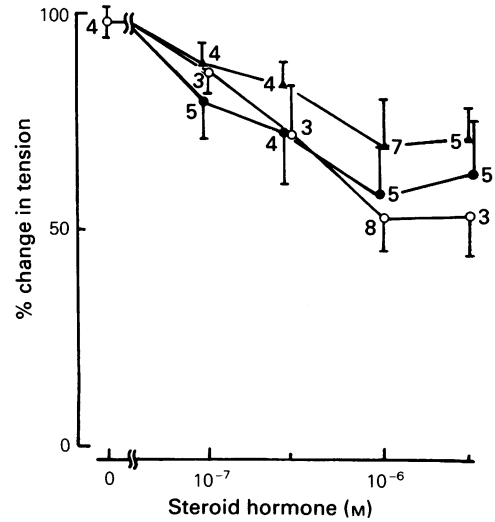

Figure 6 Concentration-response curves for the inhibitory effects of glucocorticoids on epidermal growth factor-urogastrone (EGF-URO)-induced contraction. The protocol was the same as that outlined in the legends to Figures 3 and 5. After obtaining a standard response (1.3 ± 0.1 g tension, mean ± s.e.mean for $n = 56$) to the second test dose of EGF-URO, which tension was taken as 100%, the tissues were continuously exposed to different steroid concentrations and the response to added EGF-URO was monitored at 1 h intervals, as indicated in Figure 5. The response obtained after a 2 h exposure to each concentration of steroid was used to calculate the % change in tension, relative to the standard response obtained prior to steroid treatment; resting tension was taken as 0% in each preparation. The points and bars represent the means ± s.e.mean for the number of determinations indicated beside each point: (○) hydrocortisone; (●) dexamethasone; (▲) prednisolone.

$n = 3$–8). The steroids did not alter the responsiveness of the preparation to either KCl (30 mM) or PGE_2 (30 nM) and did not alter resting muscle tension.

Since steroids have been reported to induce the synthesis of anti-inflammatory polypeptide factors in target tissues (so-called lipocortins, Flower, 1985) we examined the effect of the protein synthesis inhibitor, cycloheximide, on the inhibitory action of hydrocortisone in our preparation (Figure 7). As mentioned above, cycloheximide did not affect resting muscle tension. Not only did cycloheximide (10 μM) abolish the inhibitory effect of hydrocortisone on EGF-URO-induced contraction, but it potentiated the contractile response to EGF-URO in a time-dependent manner; the maximum effect of cycloheximide was seen at about 2 h (Figure 7). In a preparation where the response to EGF-URO was potentiated by cycloheximide, indomethacin (1 μM) abolished completely the EGF-URO-mediated contraction. Auranofin (10 μM) also inhibited EGF-URO action in the cycloheximide-treated preparations to the same degree as it did in untreated preparations (as in Figure 4).

Discussion

The main finding of our study was that a variety of steroidal and non-steroidal anti-inflammatory agents can inhibit the contractile action of EGF-URO in a non-vascular smooth muscle preparation. Our data extend considerably our previous observation that indomethacin can inhibit the contractile action of EGF-URO in a vascular smooth muscle preparation (Muramatsu *et al.*, 1985). In the present study, there was a good correlation between the order of potencies (indomethacin > naproxen > ibuprofen >

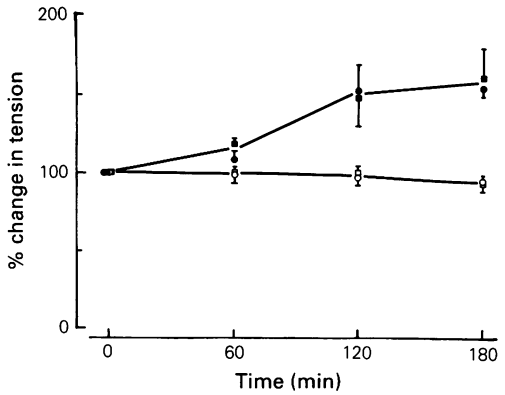

Figure 7 Effect of cycloheximide on epidermal growth factor-urogastrone (EGF-URO)-induced contraction in the presence and absence of hydrocortisone. The protocol was the same as that outlined in the legends to Figures 3 and 5. After obtaining a standard response (1.2 ± 0.1 g tension, mean ± s.e.mean for $n = 21$) to the second test dose of EGF-URO ($100\,ng\,ml^{-1}$), tissues were incubated continuously either without (○) or with added reagents: (□) 0.01% DMSO; (●) 10 μM cycloheximide; (■) 1 μM hydrocortisone plus 10 μM cycloheximide. The responses to subsequent test doses of EGF-URO ($100\,ng\,ml^{-1}$), administered for 10 min at 50 min intervals, were then measured relative to the standard response which was taken as 100% change in tension; resting tension was taken as 0%. The points and vertical bars represent the means ± s.e.mean for the following number of determinations: control 4; DMSO 7; cycloheximide 5; hydrocortisone plus cycloheximide 5.

aspirin) for the inhibition of EGF-URO-induced contraction and the previously measured inhibitory potencies of these agents (indomethacin > naproxen > ibuprofen > aspirin) in terms of blocking PGE_2 production (Annkitchen *et al.*, 1985). Thus, our results add weight to the hypothesis that an arachidonate metabolite may mediate the action of EGF-URO in some smooth muscle preparations (Muramatsu *et al.*, 1985), but not in others (Muramatsu *et al.*, 1986; Gan *et al.*, 1987). Our observations with preparations derived from guinea-pig stomach implicating a prostaglandin intermediate in the action of EGF-URO are also in keeping with the work of Chiba *et al.* (1982) who observed that EGF-URO can stimulate prostaglandin release from isolated perfused rat stomach. However, in contrast to the action of prostaglandins E_1, E_2 and $F_{2\alpha}$, which cause a non-desensitizing sustained contraction in the longitudinal stomach muscle preparation (data not shown), the effect of EGF-URO was transient and exhibited marked desensitization. The transient nature of the muscle response could be due to desensitization of the EGF-URO receptor and diffusion away from the tissue of the prostanoid responsible for causing contraction. The exact nature of the arachidonate metabolite that we suspect is involved in the EGF-URO response remains an open question which is the subject of present work in our laboratory.

Auranofin, an orally administered chrysotherapeutic agent used for treating rheumatoid arthritis, is a lipophilic compound that can react with low molecular weight thiols as well as with protein sulphhydryl groups (Crooke *et al.*, 1986). Although its mode of anti-inflammatory action is unknown, it would appear to act via mechanisms that are distinct from those of either steroidal or cyclo-oxygenase-inhibitory anti-inflammatory agents (Crooke *et al.*, 1986). The lipophilic nature of auranofin (as opposed to the hydrophilic compounds, aurothioglucose and gold sodium thiomalate) and its sulphhydryl reactivity may make it a good candidate to react directly with the membrane-localized EGF-URO receptor tyrosine kinase, which is irreversibly inhibited by sulphhydryl reagents like N-ethylmaleimide (unpublished observations). This sulphhydryl reactivity may account both for the cytotoxic action of auranofin (Mirabelli & Crooke, 1983) and for the ability of auranofin to inhibit the binding of EGF-URO to HeLa cells (Froscio *et al.*, 1987). The effect of auranofin on EGF-URO action in the smooth muscle preparation may also relate to the ability of auranofin to reduce EGF-URO binding in HeLa cells (Froscio *et al.*, 1987). The time taken for auranofin to inhibit EGF-URO action was much shorter than the time required (40 min or more) to demonstrate possible cytotoxicity in the smooth muscle preparation, as evidenced by a reduction in the contractile response to prostaglandin E_2 or to KCl; and the time course of the effect of auranofin on EGF-URO action (maximum inhibition within 20 min) is not entirely in keeping with the time taken for auranofin to have a maximum effect on EGF-URO binding in HeLa cells (40 to 60 min, Froscio *et al.*, 1987). Thus, neither cytotoxicity nor a direct effect on EGF-URO binding would appear to explain the inhibitory action of auranofin in the smooth muscle preparation; further work will be required to explain its effect.

The anti-inflammatory activity of glucocorticoids has been attributed to the induction of phospholipase-inhibitory proteins (lipocortins) that can block the cellular release of arachidonate metabolites triggered by stimuli like zymosan particles (Flower, 1985). In the work we describe here, both the time course of the inhibitory action of glucocorticoids (optimal at 2 h) and the ability of cycloheximide to reverse the inhibitory action of

glucocorticoids are consistent with the hypothesis that steroid-induced proteins (possibly lipocortins) may account for the ability of the steroidal anti-inflammatory agents to reduce EGF-URO-induced contraction. Further, the potentiating effect of cycloheximide alone on the contractile action of EGF-URO suggests that proteins related to the prostanoid-mediated action of EGF-URO may be turning over rapidly in the stomach muscle preparation, even in the absence of added glucocorticoid. Further work will be required to establish the identity of these proteins.

The structure-activity profile for the inhibitory actions of the steroids pointed to the importance of the 11β-hydroxyl and 17α-hydroxyl substituents. The importance of the 11β-hydroxyl is well recognized in terms of its absolute requirement for the anti-inflammatory activity of glucocorticoids. The lack of activity of both prednisone and cortisone (11-keto analogues of prednisolone and hydrocortisone) suggested not only that a glucocorticoid receptor was involved in the inhibitory actions of the steroids, but also that the smooth muscle preparation was not capable of reducing the 11-keto position of the steroid molecule to the active 11β-hydroxy metabolite. Despite the structure-activity data indicating that a glucocorticoid receptor was involved in mitigating the contractile response to EGF-URO, the relative potencies of the steroids (dexamethasone $\geqslant$ prednisolone $\cong$ hydrocortisone) did not agree with the relative potencies of these compounds observed both *in vivo* and with cultured cells *in vitro* (dexamethasone > prednisone $\gg$ hydrocortisone). Even given the liabilities of comparing potency ratios obtained in different response systems, the previously determined corticosteroid potency series would have predicted a 10 to 20 fold difference between the potencies of dexamethasone and hydrocortisone in terms of inhibiting the EGF-URO triggered contraction. Thus, the exact nature of the glucocorticoid receptor that is responsible for mediating the effects we have described in the smooth muscle preparation is open to question.

Although the overall physiological role for EGF-URO is uncertain, the present study adds the regulation of gastric motility to the long list of potential effects that EGF-URO may have *in vivo*. Since, like the longitudinal muscle strip we have described in the present work, many tissues (bone, fibrosarcoma cells, osteoblastic cells) appear to respond to EGF-URO by producing prostaglandins, it is likely that the inhibitory effects we have described for nonsteroidal anti-inflammatory agents, for auranofin and for glucocorticoids would also diminish the prostanoid-mediated action of EGF-URO in a wide spectrum of target tissues.

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