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Depressant action of oxybutynin on the contractility of intestinal and urinary tract smooth muscle

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Experiments were carried out in-vitro using segments of guinea-pig ileum, taenia caeci, ureter and detrusor. In the ileum, oxybutynin (30, 100 nM) competitively antagonized acetylcholine-induced contractions but did not alter those induced by histamine. Higher concentrations of oxybutynin (up to 10 μM) induced a non-competitive depression of responses to both agonists and caused a parallel shift to the right of the Ca^{2+}-induced contractions in taenia caeci strips bathed in a Ca^{2+}-free, high-K^+ medium. In the ureter, oxybutynin (1–10 μM) impaired rhythmic muscular contractions in normal medium and after $CaCl_2$ addition in Ca^{2+}-free medium. Similarly to verapamil (10, 30 μM), oxybutynin (10, 30 μM) depressed both the cholinergic and non-adrenergic, non-cholinergic components of the electrically-induced contractions of detrusor strips. It is concluded that oxybutynin has anticholinergic properties and, at higher concentrations, exerts a direct spasmolytic activity possibly mediated by blockade of the transmembrane Ca^{2+} fluxes responsible for smooth muscle contraction.

Experiments performed in-vitro or in-vivo indicate that oxybutynin (4-diethylaminobut-2-ynyl 2-cyclohexyl- 2-phenylglycolate), in addition to anticholinergic and local anaesthetic properties (Lish et al 1965), has musculotropic spasmolytic activity unrelated to tissue phosphodiesterase inhibition, as observed in rabbit detrusor by Anderson & Fredericks (1977). These properties have been exploited in clinical practice in the treatment of gut hypermotility (Rossman & Merlis 1964; Hock 1967) and, more extensively, in a number of urinary bladder disorders (Diokno & Lapides 1972; Thompson & Lauvetz 1976; Buttarazzi 1977). To date, however, no information is available on the mechanisms underlying the spasmolytic effect, even though Anderson & Fredericks (1977) hypothesized that the drug could interfere with Ca^{2+} movements across the smooth muscle cell membrane.

In this study, ileal segments and taenia caeci strips (suspended respectively in a standard medium or in a Ca^{2+}-free, high-K^+ medium) were used to re-assess the anticholinergic properties of the drug and to investigate whether oxybutynin interferes with Ca^{2+} fluxes through voltage-dependent channels (Spedding & Cavero 1984). These investigations were extended to the assessment of the action of the drug on ureteral and detrusor contractility.

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MATERIALS AND METHODS

Guinea-pigs of either sex, 350–400 g, were used. After the animal was stunned and bled, segments of distal ileum (the 10 cm proximal to the ileo-caecal junction were discarded), taenia caeci, ureter and urinary bladder (detrusor) were dissected and immersed in appropriate solutions (see below) at 36 °C continuously bubbled with a mixture of 95% O_2 + 5% CO_2.

Ileal preparations

Pieces of distal ileum 3 cm in length were mounted isotonically (load 1 g) in a 20 mL organ bath containing Tyrode solution. After a 30 min equilibration period, cumulative concentration-response curves for both acetylcholine (0·016–160 μM) and histamine (0·016–16 μM) were constructed in the absence and in the presence of various concentrations of oxybutynin (0·03–10 μM) added in the bath 5 min before agonist administration. Contractile responses to graded concentrations of acetylcholine and histamine were expressed as a percent of the maximum response produced by the respective agonist. Cumulative concentration–response curves to acetylcholine and histamine were fully reproducible and therefore no sign of tachyphylaxis was observed in spite of repetitive agonist administrations.

Taenia caeci strips

Strips of taenia (2·5–3 cm in length) dissected from the caecum were mounted isotonically (load 1 g) in a

20 mL organ bath containing Ca^{2+}-free, K^+-depolarizing Tyrode solution. After a 30 min equilibration period, cumulative concentration–response curves to $CaCl_2$ (0·03–3 mM) were obtained by increasing the Ca^{2+} concentration at 3 min intervals. The 100% response was taken as the maximum contractile response of the strip during the second concentration–response curve, and all subsequent contractions were expressed as a percentage of this value (Spedding 1982). Cumulative concentration–response curves were repeated in the presence of oxybutynin (1–10 μM) added to the bath 20 min before $CaCl_2$ administration. Dose ratios were calculated at the EC50 level with and without oxybutynin. The apparent pA_2 value was calculated by the method of Arunlakshana & Schild (1959).

Ureteral preparations

Segments of ureter, approximately 4 cm in length, with the oral end cut just above the pelvi-ureteral junction (which is considered to be the primary site of origin of rhythmic muscular contractions: Qayum 1978), were mounted in a 50 mL organ bath. After the lumen was filled with Tyrode solution, the proximal end of the ureter was occluded and connected by a thread to an isotonic lever (load 1 g), while the distal end was connected to a pressure transducer. Rhythmic muscular contractions (detected as variations in length of the segment and as intraluminal pressure changes) took place when intraluminal pressure was increased up to 8 cmH_2O. These contractions could be recorded for several hours, usually with no decline in activity. The depressant action of oxybutynin (1–10 μM) on ureteral contractile activity was evaluated by exposing the tissue for 10 min to a single concentration of the drug. The post-drug magnitude of contractions was expressed as percentage of the control contractions.

Detrusor strips

Detrusor strips (10 × 2 mm) were prepared by dissecting the urinary bladder along the minor axis. After removal of the mucosa, the preparation was suspended isometrically (tension 0·5 g) in an organ bath (3 mL) filled with Tyrode solution containing guanethidine (3·4 μM). Electrical field stimulation was achieved by silver electrodes delivering pulses of 0·5 ms duration at a voltage of 25 V and at frequencies ranging from 0·3 to 50 Hz. Nerve stimulation was maintained until the tension of the strip reached a maximum and declined. Frequency–response curves were obtained non-cumulatively in the absence and in the presence of antagonists after a 90 min stabilization period. All contractions were expressed as a percentage of the response obtained with stimulation at 50 Hz at the beginning of each experiment.

Statistical analysis

Student's *t*-test was used for comparisons of mean values. Values in the text refer to means ± s.e.m.

Solutions and drugs

The Tyrode solution had the following composition (mM): NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·04, $NaHCO_3$ 11·9, NaH_2PO_4 0·4, glucose 5·5. The composition of the Ca^{2+}-free, high-K^+ Tyrode solution was (mM): NaCl 97, KCl 40, $NaHCO_3$ 11·9, NaH_2PO_4 0·4, glucose 5·5. The following drugs were used: oxybutynin hydrochloride (Scharper), acetylcholine chloride (Sigma), histamine dihydrochloride (Sigma), atropine sulphate (BDH), verapamil (Knoll), guanethidine sulphate (Ciba) and tetrodotoxin (Sankyo).

RESULTS

Ileal preparations

Oxybutynin (30, 100 nM) dose-dependently shifted to the right the concentration–response curve to acetylcholine (Fig. 1A) without depressing the maximum response. The antagonism was reversible on washing, the dissociation constant K_B (calculated from the equation $K_B = B/(X - 1)$ where B = antagonist concentration; X = dose ratio) at 30 nM being $1·3 \times 10^{-8}$ M. These concentrations of oxybutynin did not modify the concentration–response curve to histamine (Fig. 1B). However, when the concentration of oxybutynin was increased to 3–10 μM, a non-competitive depression of both acetylcholine and histamine contractions was observed.

Taenia caeci strips

Pretreatment of taenia caeci strips with oxybutynin (1–10 μM) caused a parallel displacement to the right of the Ca^{2+} concentration–response curves (Fig. 2). Antagonism was partially or even completely reversible depending on the duration of washing (complete reversibility was usually obtained by several changes of bath fluid and drop by drop washing for 60–90 min). The slope of the Arunlakshana & Schild plot (−1·11 ± 0·12) was not significantly different from −1, the value expected for competitive antagonism. The apparent pA_2 value was 6·23 ± 0·04 (data from Fig. 2).

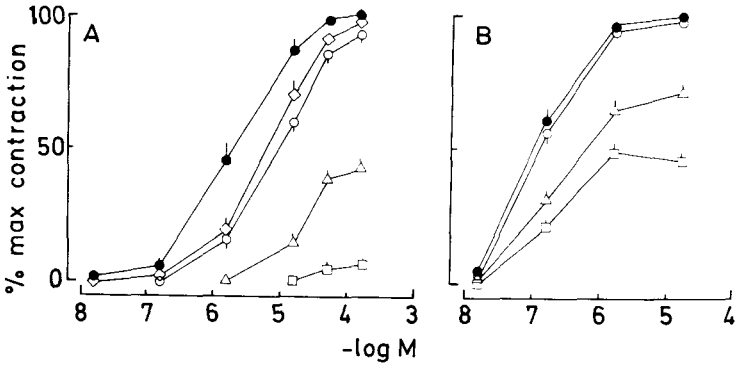

FIG. 1. Log molar (M) concentration–response curves for acetylcholine (A) and histamine (B) in contracting the longitudinal muscle of the guinea-pig ileum. A and B: control responses (●); responses in the presence of 0·03 (◇), 0·1 (○), 3 (△) and 10 μM (□) oxybutynin. Each symbol represents the mean ± s.e.m. of 4–6 experiments.

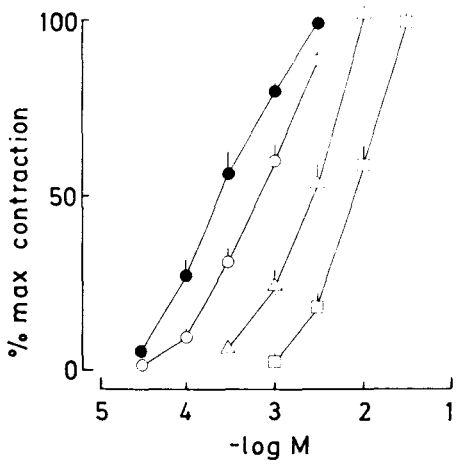

FIG. 2. Log molar (M) concentration–response curves for $CaCl_2$ in contracting the guinea-pig taenia caeci bathed in Ca^{2+}-free, high-K^+ medium. Control responses (●); responses in the presence of 1 (○), 5 (△) and 10 μM (□) oxybutynin. Each symbol represents the mean ± s.e.m. of 6 experiments.

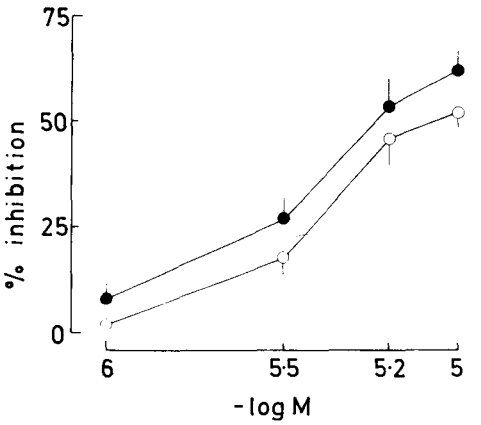

FIG. 3. Log molar (M) concentration of oxybutynin in inhibiting longitudinal contractions (○) and intraluminal peak pressure changes (●) in ureteral preparations. Oxybutynin (all concentrations except 1 μM) significantly depressed ($P < 0·05$ or better) both mechanical events. Each symbol represents the mean ± s.e.m. of 6 experiments.

Ureteral preparations

Ureteral rhythmic contractions were associated with intraluminal pressure changes synchronous with variations in length of the preparation. The mean frequency of contractions was 6·2 ± 0·6 min^{-1} (n = 20). Each preparation usually maintained consistently its own frequency for a prolonged period of time (90–120 min). Tetrodotoxin (0·7 μM, n = 5) had no major influence on ureteral activity, causing only a slight increase (approximately 15%) of the frequency of both mechanical events considered. Oxybutynin (1–10 μM) dose-dependently depressed the amplitude of longitudinal contractions and peak pressure (Fig. 3). As far as the frequency of mechanical events is concerned, lower oxybutynin concentrations (1, 3 μM) were associated with no change or with a slight increase in frequency (10–20%, n = 6), while at higher concentrations (6, 10 μM) a slowing frequency (5·8 ± 0·6 min^{-1} vs 4·0 ± 0·5 min^{-1}, n = 6, $P < 0·05$, and 6·3 ± 0·7 min^{-1} vs 2·4 ± 0·3 min^{-1}, n = 6, $P < 0·01$) was observed. Change of the normal Tyrode to a Ca^{2+}-free solution caused the contractile activity to stop. After addition of $CaCl_2$, contractions consistently reappeared (a 1 mM $CaCl_2$ concentration was usually effective). Pretreatment for 5 min with oxybutynin (5 μM) partially prevented the effect of 1 mM $CaCl_2$ (Fig. 4). In the presence of oxybutynin, control contractions could not be resumed completely (in both frequency and amplitude), even after administration of 2–5 mM $CaCl_2$.

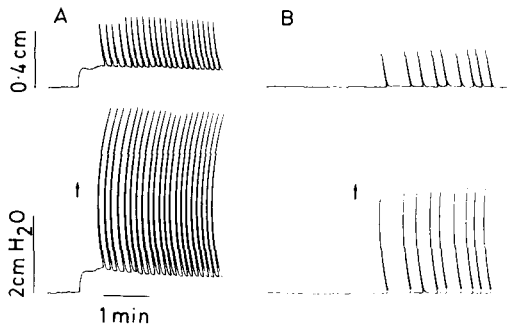

FIG. 4. Ureteral motility recorded after addition of 1 mM $CaCl_2$ (at the arrow) to a Ca^{2+}-free medium. A and B from top to bottom: length (cm) and pressure (cmH_2O) changes of the preparation during rhythmic contractions. A: Control responses. B: same as A but in the presence of 5 μM oxybutynin. In the presence of oxybutynin an approximately 30–40% inhibition of the amplitude of both mechanical events was observed, while the frequency of contractions was reduced to 40–70% of control value (n = 3).

Detrusor strips

In the electrically stimulated detrusor strips oxybutynin (10 μM) was shown to inhibit contractions evoked by 10 and 30 Hz stimulation only (Fig. 5). The magnitude of this inhibitory effect was similar to that obtained with 2 μM atropine which also did not alter the contractions elicited by lower frequencies (data not shown). Depression of contractions over the entire range of frequencies was obtained by using a three-fold greater oxybutynin concentration (30 μM). All these contractions were also dose-dependently depressed by 10 and 30 μM verapamil (Fig. 5).

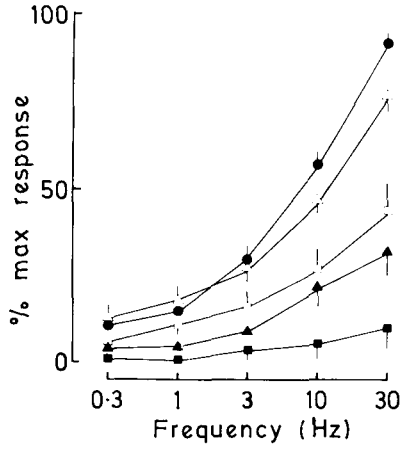

FIG. 5. Frequency-dependent excitation of detrusor muscle by field stimulation. Control contractions are indicated as closed circles (●). Contractions in the presence of 10 and 30 μM oxybutynin are represented by open (△) and closed triangles (▲) respectively. Open (□) and closed squares (■) indicate contractions in the presence of 10 and 30 μM verapamil. Each symbol represents the mean ± s.e.m. of 8 experiments. Significant differences in the magnitude of the responses were found in the presence of 10 μM oxybutynin at 10 and 30 Hz ($P < 0.05$) and in the presence of oxybutynin (30 μM) and verapamil (10, 30 μM) at all frequencies used ($P < 0.01$). For clarity, s.e.m. are not shown on all points.

DISCUSSION

Our results confirm previous data and add new information on the mode of action of oxybutynin in intestinal and extraintestinal smooth muscle. At relatively low concentrations (30, 100 nM), oxybutynin behaved as a competitive anticholinergic in the ileum. This is consistent with recent findings showing that oxybutynin binds specifically to muscarinic receptors in different tissues (Nilvebrant & Sparf 1986). Antagonism was specific in that the concentration–response curve to histamine was unaltered by these oxybutynin concentrations. Higher concentrations of the drug (3, 10 μM) were associated with non-competitive depression of both acetylcholine and histamine contractions.

These findings are essentially similar to those described in the rabbit isolated detrusor by Fredericks et al (1975) and Anderson & Fredericks (1977). Therefore, within the micromolar range of concentrations the spasmolytic action of oxybutynin appears to be independent of the contracting agent used, and probably involves interference with the mechanisms of muscular contractions. Previous findings (Anderson & Fredericks 1977) have demonstrated that the direct spasmolytic effect of oxybutynin is unrelated to tissue phosphodiesterase inhibition. Our results in the K^+-depolarized taenia caeci suggest that this effect may be due to Ca^{2+}-antagonism. In fact, the oxybutynin (1–10 μM)-induced parallel dose-dependent displacement to the right of the concentration–response curve to $CaCl_2$ is typical of a relatively weak calcium entry blocker. Since oxybutynin has a definite local anaesthetic action (Lish et al 1965), it is possible that the inhibitory effect on Ca^{2+}-induced contractions observed is related to this property (Hay & Wadsworth 1982; Spedding 1983, 1986).

The Ca^{2+}-antagonist effect of oxybutynin may explain its action on ureteral contractile activity. Indeed, oxybutynin depressed not only rhythmic contractions elicited in normal medium but also those evoked by $CaCl_2$ administration in a Ca^{2+}-free medium. This inhibitory action probably took place at muscular level, since ureteral contractions were tetrodotoxin-resistant and therefore independent of nervous drive. Early studies in the guinea-pig de-

trusor had suggested that contractions induced by electrical field stimulation were largely atropine-resistant and mediated mainly by non-adrenergic, non-cholinergic innervation (Chesher & Thorp 1965; Ambache & Zar 1970). Subsequent studies, however, have described a cholinergic component in these contractions (Krell et al 1981; MacKenzie & Burnstock 1984). In our study in the detrusor muscle, we found atropine effective in antagonizing only contractions evoked at frequencies equal to or above 10 Hz, in agreement with MacKenzie & Burnstock (1984). As far as the action of oxybutynin in this model is concerned, 10 μM oxybutynin was found to depress the contractile response at 10 and 30 Hz stimulation by the same extent as atropine at a concentration of 2 μM (our result) or 1·4 μM (MacKenzie & Burnstock 1984). Therefore, in this preparation, the action of 10 μM oxybutynin could be explained in terms of anticholinergic effects, even though a possible contribution by Ca^{2+}-antagonistic properties (seen at this concentration in the intestinal and ureteral muscle) cannot be excluded. Similarly to verapamil, higher oxybutynin concentrations depressed the non-adrenergic, non-cholinergic component of the response, probably by a direct action on smooth muscle (Kaplita & Triggle 1983; Beattie et al 1986).

The relaxant action of oxybutynin on the urinary bladder, with a consequent increase in bladder capacity, is exploited clinically for the treatment of vesical disorders such as neurovesical reflex activity, uninhibited bladder, enuresis and primary bladder spasm (Thompson & Lauvetz 1976; Paulson 1978; Hilwa & Perlmutter 1978; Moisey et al 1980). Both anticholinergic and direct spasmolytic properties may contribute to this action. It is known, in fact, that the nerve-mediated contractile activation of the human detrusor is also partially resistant to atropine (Cowan & Daniel 1983) and this atropine-resistant component of the response can be blocked by nifedipine (Sjögren et al 1982). Thus, a drug combining anticholinergic and calcium blocking properties should be valuable for the treatment of bladder overactivity (Andersson & Ulmsten 1980; Andersson 1984). Further studies, however, are required to establish whether administration of therapeutic doses of oxybutynin in man can produce sufficiently higher concentrations for a direct spasmolytic effect to take place in the clinical situation.

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