Difference in the mechanisms by which acetylcholine and histamine interact with Ca^{2+} to contract the rabbit taenia coli

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It is a current view that in smooth muscle, as in striated muscle and myocardium, a rise in free intracellular Ca^{2+} activates contraction. This rise in Ca^{2+} which leads to muscle contraction may result from one or several of the following mechanisms: (1) increase in the membrane permeability to extracellular Ca^{2+} which enter the cell; (2) reduction of the active mechanism which controls the Ca-efflux; (3) acceleration of Ca-release from intracellular sites of storage.

Therefore the manner in which acetylcholine and histamine depend on Ca^{2+} to elicit a contractile response in rabbit taenia coli has been investigated. It appears that each of the agonists interacts with Ca^{2+} distinctively.

Two groups of experiments were made in this study. Male rabbits, 1·8 to 2·2 kg, were killed, the taenia coli isolated and a piece (2 to 4 cm) was suspended in 20 ml organ bath filled with Locke Ringer solution kept at 32° and bubbled with air. Responses of the smooth muscle to histamine and acetylcholine were recorded isotonically. The agonists were added cumulatively to the bath fluid and the maximum response was obtained in the concentration of 3×10^{-4} M of each agonist. Normal Locke Ringer solution used had a following composition (mM): NaCl 154, KCl 5·6, $CaCl_2$ 2·2, $MgCl_2$ 2·1, $NaHCO_3$ 5·9 and glucose 2·8. Experiments were also carried out in the Ca-free Locke Ringer solution made by omitting $CaCl_2$ from the normal Locke Ringer solution.

In the second experiment male rabbits, 1·8 to 2·2 kg, were killed and taenia coli was immediately removed and washed with ice-cold 0·25 M sucrose solution. The isolated taenia was cut into small pieces and homogenized in 9 volumes of ice-cold 0·25 M sucrose containing 10 mM tris-HCl buffer (pH 7·4) in a Polytron (P.T.-10, Brinkman Instruments) with the rheostat setting 6 for 5 s and 7 for further 5 s. The homogenate was centrifuged for 20 min at 9000 *g* and the supernatant centrifuged at 15 000 *g* for 20 min. Centrifugation of the supernatant at 40 000 *g* for 90 min resulted in a pellet which was used as the microsomal fraction. All the procedures were performed at 0°.

Ca-uptake experiments were performed at 32° in the incubation medium which contained (mM) histidine buffer 30 (pH 7·4), KCl 100, $MgCl_2$ 5, potassium oxalate 5, sodium azide 5, ATP tris 3 and $CaCl_2$ 0·02 plus $^{45}CaCl_2$ (0·2 μCi ml^{-1}) according to Takayanagi, Yamashita & others (1977). The release of microsomally incorporated Ca was examined after microsomes were suspended in the incubation medium for a period of 10 min in order to subject microsomes to Ca^{2+}. The experiments were started at 32° by 10-fold dilution of the suspension of microsomes with the incubation medium from which $CaCl_2$ and potassium oxalate were omitted and stopped by filtration of the suspension through a Millipore filter (HAWP02500) according to Baudouin-Legros & Meyer (1973). The amount of Ca^{2+} on the microsomes at different times after dilution was determined by liquid scintillation spectrometry, after the filters had been dissolved in toluene scintillator (Tomiyama, Takayanagi & Takagi, 1975). The values were corrected for the ^{45}Ca remaining on the filters in the absence of the microsomes.

Microsomal protein concentration was determined by the method of Lowry, Rosebrough & others (1951), using bovine serum albumin as the standard. The amount of protein contained in the incubation medium was 400 to 500 μg ml^{-1}.

The mechanical responses (mean ± s.e. of 6 experiments) to acetylcholine (5×10^{-5} M) and to histamine (5×10^{-5} M) were respectively 77 ±2% and 78 ±3% of the maximum response. Atropine (3×10^{-6} M) completely abolished the response to acetylcholine (5×10^{-5} M) but did not influence that to histamine (5×10^{-5} M). After 10 min incubation of the taenia coli with the Ca-free Locke Ringer solution the responses (mean ±s.e. of 6 experiments) of the smooth muscle to acetylcholine (5×10^{-5} M) and histamine (5×10^{-5} M) in the Ca-free Locke Ringer solution were 46 ±8% and 28 ±4% of those observed in the normal Locke Ringer solution. Two values are significantly different from each other at $P < 0·05$. The response of rabbit taenia coli to histamine is therefore more dependent on the presence of external Ca^{2+} than the response to acetylcholine.

Electron micrographs of the 40 000 *g* pellet of rabbit taenia coli used showed that this fraction consisted of

Table 1. *Effects of acetylcholine and histamine on Ca-release from microsomes of rabbit taenia coli.* Each value (mean ±s.e. of 5 experiments) is expressed in %. The amount of Ca^{2+} in microsomes at 0 time had been considered as 100%.

Incubation time	0	30 s	90 s	240 s
Control	100	96 ± 1·3	87 ± 0·9	78 ±1·2
Histamine (5×10^{-5} M)	100	92 ± 0·8	86 ± 1·3	77 ± 2·0
Acetylcholine (5×10^{-5} M)	100	89 ± 0·6*†	81 ± 1·0*†	69 ±1·8*‡

The paired *t*-test was performed.

* Significantly different from a control value at a corresponding incubation time ($P < 0·01$).

† Significantly different from the value in the presence of histamine ($P < 0·05$).

‡ Significantly different from the value in the presence of histamine ($P < 0·02$).

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Table 2. *Effect of atropine on Ca-release induced by acetylcholine.* Each value (mean ±s.e. of 5 experiments) is expressed in %. The amount of Ca^{2+} in microsomes at 0 time has been considered as 100%.

Incubation time	0	30 s	90 s	240 s
Control	100	98 ± 1·0	93 ± 1·6	86 ± 1·0
Acetylcholine (5 × 10^{-5} M)	100	93 ± 0·8	86 ± 1·2	81 ± 1·4
Atropine (3 × 10^{-6} M)	100	99 ± 201	92 ± 1·8	87 ± 1·9
Acetylcholine (5 × 10^{-5} M) + atropine (3 × 40^{-6} M)	100	96 ± 1·5*	91 ± 1·3*	87 ± 2·0*

The paired *t*-test was performed.
* Significantly different from the value in the presence of acetylcholine alone ($P < 0·05$).

vesicular structures. Most vesicles consisted of smooth muscle membranes. There were no intact mitochondria. The microsomal fraction used contained high concentrations of markers of plasma membrane, such as acetylcholinesterase, Na-, K-ATPase and 5′-nucleotidase.

The rate of Ca-release was significantly increased by acetylcholine (5 × 10^{-5} M) at all incubation times (Table 1). This increase was blocked by atropine (3 × 10^{-6} M) which had no significant effect on Ca-release in the absence of acetylcholine (Table 2). Thus release of Ca^{2+} induced by acetylcholine seems to be due specifically to acetylcholine receptors. However, histamine did not increase Ca-release significantly (Table 1). Baudouin, Meyer & others (1972) and Baudouin-Legros & Meyer (1973) demonstrated that angiotensin II increased the release of ^{45}Ca from the microsomes of rabbit aorta, while analogues of angiotensin II devoid of intrinsic activity failed to alter the release of ^{45}Ca. The present results concerned with the release of ^{45}Ca and the findings of Baudouin & others (1971) and Baudouin-Legros & Meyer (1973) suggest that the mechanical response induced by the drugs which affect the smooth muscle tone is, at least partially, initiated by a translocation of Ca^{2+} from the binding stores in cellular membranes. Furthermore the results in the present study suggest that the initial contraction of the rabbit taenia coli induced by acetylcholine is more dependent on the release of Ca from the cellular sites of storage than that induced by histamine, while the response to histamine is the more dependent on external Ca^{2+}.

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Mercury content of medicinal lithium preparations

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Lithium salts used prophylactically to attenuate or prevent recurrences of manic-depressive disease, are given to patients for long periods (Baastrup & Schou, 1967, Schou, 1976). Because of this the preparations should not contain impurities that might accumulate and exert toxic actions. Ordinary lithium hydroxide is bought for the extraction of ^{6}Li, and the residue is then made available for general use. Analysis of ^{6}Li depleted materials reveals that there is a significant increase of the content of mercury as a result of the ^{6}Li extraction procedure used (Lithium Corporation of America, private communication, 1976). For this reason some producers avoid using ^{6}Li-depleted material for lithium products intended for medicinal purposes. Other producers may not be equally cautious. We have therefore analysed 23 preparations from 14 countries using an unpublished method by Nielsen Kudsk for determination of total mercury. This is based upon a modification of the analytical principle described by Schütz (1969).

Tablets or capsules were ashed by combustion at 1000° in a quartz tube in a stream of pure oxygen, and mercury liberated together with the combustion gas was trapped in an acid potassium permanganate solution. The amount of mercury collected was determined spectrophotometrically by atomic absorption, after reduction with stannous chloride and liberation from solution by shaking and aeration with an atmospheric air stream, using a Varian atomic absorption spectrophotometer AA-6, equipped with a 17 cm gas cuvette having quartz windows.

The detection limit is about 1·5 ng mercury per

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