# Possible involvement of a cholinergic mechanism in calcium-induced contractions of chick oesophagus

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While working out the mechanism of adrenaline-induced contractions on the chick post-crop oesophagus, especially in relation to $Ca^{2+}$ involvement, we observed a contractile effect of $CaCl_2$ on this tissue in a non-depolarized state. We have therefore investigated the possible mechanism of $CaCl_2$ contractions on this preparation.

White leghorn chicks of either sex, 1–2 weeks old, were killed by a blow on the head. The oesophagus was separated from the crop and cleaned. The tissue pieces were suspended in organ baths of 10 ml volume containing Krebs-Henseleit solution (NaCl 6·95, KCl 0·34, $CaCl_2$ 0·28, $KH_2PO_4$ 0·162, $MgSO_4$ 0·294, $NaHCO_3$ 2·1 and glucose 2·0 g litre$^{-1}$), maintained at 31–32 °C and continuously aerated with a $CO_2$ 5%–$O_2$ 95% mixture. The longitudinal muscle contractions were recorded isotonically on a slow moving kymograph. An interval of 45 min was maintained between two successive doses of drugs. To depolarize the tissue, NaCl in the Krebs-Henseleit solution was replaced by an equimolar amount of KCl. Several experiments were conducted on the tissues kept at 4 °C for 72 h.

The drugs used were calcium chloride ($CaCl_2$), acetylcholine chloride, carbachol, 5-hydroxytryptamine creatinine sulphate, histamine dihydrochloride, physostigmine salicylate, neostigmine methyl sulphate, verapamil, atropine sulphate, cyproheptadine hydrochloride, mepyramine maleate, pentolinium tartrate, (+)-tubocurarine chloride, papaverine hydrochloride, polysorbate 80 (Tween–80) and lignocaine hydrochloride.

$CaCl_2$ (1–2 mg ml$^{-1}$) produced dose-dependent contractions on the tissue (Fig. 1), bathed in normal $K^+$ solution, the onset of contraction being delayed by about 45 s to 1 min after administration. Repeated exposure of the tissue to calcium resulted in tachyphylaxis, when the time interval between two subsequent doses was less than 30 min. However, by increasing the time interval to 45 min, consistent contractile responses were obtained. Several experiments were done at different concentrations of $CaCl_2$ (0, 70 and 280 mg litre$^{-1}$) in the Krebs-Henseleit solution wherein the magnitude of the contractile responses of the tissues to $CaCl_2$ remained unaffected.

Contractions in response to calcium were challenged by a series of antagonists. Verapamil (10 μg ml$^{-1}$), a calcium antagonist, effectively abolished (80%)/reversed (20% of the experiments) the calcium-induced contractions and also inhibited the contractile responses of acetylcholine (0·05 to 0·1 μg ml$^{-1}$), carbachol (0·03–05 μg ml$^{-1}$) and 5-hydroxytryptamine (5-HT) (0·1–1 ng ml$^{-1}$). Pre-incubation of the tissue with atropine (1 μg ml$^{-1}$) sufficient to abolish the acetylcholine or carbachol responses markedly diminished (Fig. 1) and subsequently abolished the calcium contractions. Neostigmine or physostigmine (1 μg ml$^{-1}$) potentiated the calcium- and carbachol-induced contractions significantly (Fig. 2) and further facilitated the rapid recovery of the contractile responses of the tissues already blocked with atropine.

Specific antagonists of histamine $H^1$-receptors (mepyramine, 1 μg ml$^{-1}$) and 5-HT receptors (cyproheptadine, 0·5 μg ml$^{-1}$) had no influence on the calcium contractions. Also, neither pentolinium (1 μg ml$^{-1}$) nor (+)-tubocurarine (2 μg ml$^{-1}$) had any apparent effect on the calcium responses. Lignocaine (50 μg ml$^{-1}$), a membrane stabilizer, however, potentiated the calcium contractions. On the oesophageal preparations, cooled at 4 °C for 72 h, $CaCl_2$ showed either no response or induced small relaxations. However, on depolarization, graded and reversible contractile responses to calcium were obtained on these tissues.

As non-depolarized smooth muscle has been shown to contract in response to calcium, we examined the relative sensitivity of polarized and depolarized chick oesophagus for the magnitude of calcium-induced contractions. Surprisingly, the responses to calcium in 'KCl-Krebs-Henseleit' were of less magnitude than those in a non-depolarized tissue preparation bathed in normal $K^+$-solution.

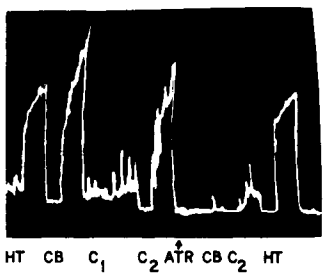


FIG. 1. The effect of atropine on 5-HT, carbachol and calcium-induced contractions on the chick oesophagus. At HT, 5-hydroxytryptamine (0·1 ng ml$^{-1}$); at CB, carbachol (0·05 μg ml$^{-1}$) and at $C_1$ and $C_2$, calcium chloride (1 and 2 mg ml$^{-1}$) were added to the bath. At ATR, atropine was added to give a bath concentration of 1 μg ml$^{-1}$. The time interval between two successive doses was 45 min.

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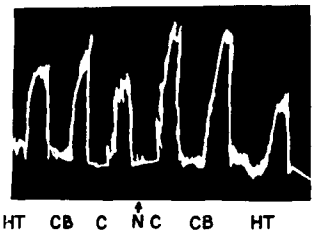


FIG. 2. The effect of neostigmine on the responses of 5-hydroxytryptamine, carbachol and calcium chloride on the chick oesophagus. At HT, 5-hydroxytryptamine (0·1 ng $ml^{-1}$); at CB, carbachol (0·05 $\mu g$ $ml^{-1}$) and at C, calcium chloride (1 mg $ml^{-1}$) were added to the bath. At N, neostigmine was added to give a bath concentration of 1 $\mu g$ $ml^{-1}$. Time interval between two successive doses was 45 min.

The presence of excess $Ca^{2+}$ decreases spontaneous muscle tone (Ambache 1946) and reduces the spike frequency (Holman 1958) and $K^+$ efflux (Weiss & Hurwitz 1962). Calcium (0·9–1·8 mg $ml^{-1}$) produces dose-dependent relaxation of the rabbit intestine (Ambache 1946) and of chick ileum, guinea-pig ileum and rabbit duodenum (unpublished work). On the other hand, in a state of depolarization, the mobilization of extracellular $Ca^{2+}$ takes place, thus during a depolarization maintained with $K^+$-excess physiological solution, influx of the extracellular $Ca^{2+}$ triggers the contraction (Chapman 1966). The present observations of dose-dependent contractions by $CaCl_2$ in chick oesophagus, a tissue exhibiting autorhythmicity, are in contrast to the general behaviour of smooth muscle preparations to calcium. However, this finding substantiates the earlier observation on an isolated artery preparation in which, in the presence of normal $K^+$, high $Ca^{2+}$ produced a small but significant contraction (Hinke et al 1964).

The delayed onset of contraction and subsequent development of tachyphylaxis suggest a possible involvement of some mediator in the initiation of $CaCl_2$ responses. The abolition of calcium contractions by atropine and its marked potentiation by physostigmine or neostigmine strongly suggest a possible cholinergic link. This view is further strengthened by the finding that the AChE activity of chick oesophagus is altered in the presence of calcium (unpublished work). However, we are unable to explain the phenomenon of carbachol potentiation by neostigmine. The method of cooling provides a means of obtaining a smooth muscle preparation free from the neural influences (Ambache 1946). Hence, the abolition or reversal of the contractile responses of $CaCl_2$ on cooling implicates participation of neural elements in the mediation of calcium responses. The involvement of other mediators like histamine and 5-HT is ruled out since mepyramine and cyproheptadine respectively did not alter the responses. The resistance of the calcium contractions to pentolinium or (+)-tubocurarine eliminates the ganglionic involvement.

Verapamil, a blocker of the $Ca^{2+}$ channels, prevents the entry of extracellular $Ca^{2+}$ into the smooth muscle cells (Fleckenstein 1977). In the present study, it abolished or reversed the calcium contractions and inhibited those of acetylcholine, carbachol and 5-HT. Also, non-specific smooth muscle blockers like papaverine and Tween-80 inhibited the calcium responses.

Replacement of normal Krebs-Henseleit solution with that of $K^+$-excess solution resulted in the contracture of the oesophagus which then slowly relaxed to the base line; the addition of calcium at this stage produced dose-dependent and reversible contractions of the tissue. This is indirect evidence that the tissue bathed in normal $K^+$ was not in a state of depolarization. Reduction in external calcium is reported to produce partial depolarization (Burnstock & Straub 1958) of the smooth muscle membrane and here the tissue contracts to calcium as in the case of depolarization. However, no appreciable changes in the magnitude of calcium contractions were observed when the experiments were conducted at different concentrations of calcium in Krebs-Henseleit solution.

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