Calcium and the contractile response to prostaglandin in the smooth muscle of guinea-pig stomach

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Summary. In the longitudinal smooth muscle of guinea-pig stomach, verapamil (10^{-5} M) which showed marked suppression of high K-induced contractures, did not suppress the contractile response to PGE_1 (1.5×10^{-9} to 10^{-6} M) markedly. These results suggest that the contractile mechanism of PGE_1 in guinea-pig stomach may mainly depend on a release of bound Ca in the cell and partly depend on a Ca influx from the extracellular origin.

Introduction. In smooth muscle, the Calcium (Ca) which results from extracellular and/or intracellular origin(s) is necessary for the contractile process[1-3]. Prostaglandin (PG) produced contraction of the isolated longitudinal smooth muscle of gastrointestine[4], and it has been considered that this stimulating action might depend on an increase of Ca ion permeability through the muscle membrane[5].

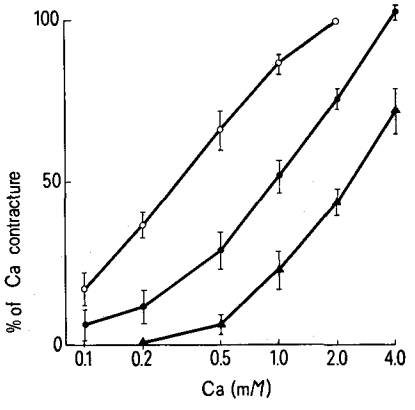

Fig. 1. Effect of verapamil on Ca contracture in longitudinal smooth muscle of guinea-pig stomach. ○—○, control; ×—×, verapamil 2×10^{-8} M; △—△, verapamil 10^{-7} M. Ca contracture at Ca 2 mM was taken as 100%. Each point is the mean of 5 experiments and vertical bar is ± S. E. of mean.

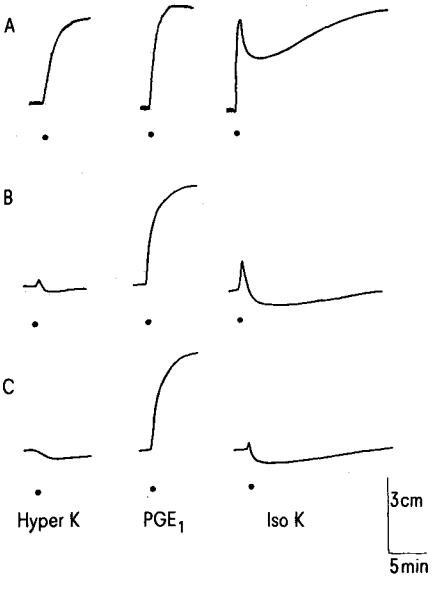

Fig. 2. Effect of verapamil on the contractile responses to PGE_1 (1.5×10^{-7} M), hypertonic K (Hyper K) and isotonic K (Iso K) in longitudinal smooth muscle of guinea-pig stomach. *A* control, *B* verapamil 10^{-6} M, *C* verapamil 10^{-5} M.

Golenhofen and Wegner[6], however, reported that there existed 2 different Ca activation mechanisms in smooth muscle of guinea-pig stomach, one of which was blocked by treatment with Ca antagonists (e.g. verapamil and its methoxy derivative). These antagonists have been reported to block spike discharge and high K-induced contracture in smooth muscle[7,8].

The present study was carried out to clarify the origin of Ca on the contractile response to PGE_1 by treatment with verapamil in longitudinal smooth muscle of guineapig stomach.

Methods. Guinea-pigs of 300–600 g weight were killed by a blow on the head and bled. The stomach was removed and a muscle strip (20 mm length and 2 mm width) was cut parallel to the longitudinal muscle layer of the corpus. The muscle was separated from the mucosal membrane and immersed in 10 ml of the bath solution containing NaCl 125, KCl 5.7, $CaCl_2$ 2.0, $MgCl_2$ 0.5, glucose 11.5 and $NaHCO_3$ 15.0 mM. The bath solution was kept at 37 ± 0.1 °C and gassed with 5% CO_2 in O_2, pH 7.2. Movements of the muscle were recorded isotonically on a kymograph (magnification is 8 times and the load on muscle is 0.5 g).

Each hypertonic and isotonic K contracture was obtained by the addition of KCl (30 mM) to the bath solution and by replacing the bath solution with the isotonic K solution, respectively. Ca contracture was gained by the addition of $CaCl_2$ to the Ca(–) K solution. Verapamil added to the bath solution 10 min prior to agonist in all experiments. Drugs: verapamil (supplied by the Eisai Co.), PGE_1 (supplied by the Ono Co.) and acetylcholine (Daiichi) were used.

Results and discussion. The dose-response curve of Ca contracture showed a parallel shift to the right by treatment with verapamil (2×10^{-8} and 10^{-7} M) (figure 1). Both hypertonic and isotonic K contractures were suppressed by verapamil (10^{-5} M) markedly (figure 2). It has been reported that verapamil and its methoxy derivative were very active antagonists for Ca uptake in smooth muscle[3,9], skeletal muscle[10] and heart muscle[10,11].

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The present observations revealed that verapamil was a strong antagonist for Ca influx in guinea-pig stomach, since the calculated pA_2 value for Ca contracture was 7.92. The mechanism of PG-induced contraction in gastrointestinal smooth muscle was considered to depend on an increase of Ca ion permeability through the muscle membrane, since PGE_1 potentiates Ca contracture in guinea-pig stomach[5] and increases spike discharge in guinea-pig ileum[12] and taenia coli[13], and PG-induced contraction is related to the extracellular Ca ion in rabbit duodenum[13].

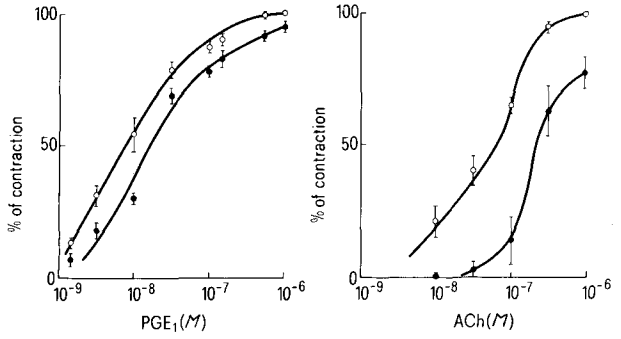

Fig. 3. Effect of verapamil on the contractile responses to PGE_1 (1.5×10^{-9} to 10^{-6} M) and ACh (10^{-8} to 10^{-6} M) in longitudinal smooth muscle of guinea-pig stomach. ○—○, control; ×—×, verapamil 10^{-5} M. Each contractile response to PGE_1 (10^{-6} M) and ACh (10^{-6} M) was taken as 100%, respectively. Each point is the mean of 5 experiments and vertical bar is ± S. E. of mean.

In the present experiments, however, verapamil (10^{-5} M) which produced marked suppression of high K-induced contractures, showed only small reduction of PGE_1 (1.5×10^{-7} M)-induced contraction (figure 2). Figure 3 showed the effect of verapamil (10^{-5} M) on dose-response curves of PGE_1 and acetylcholine (ACh), indicating that verapamil caused more significant reduction of ACh-induced contraction than that of PGE_1. The calculated pA_2 values for PGE_1 and ACh were 5.06 and 5.33, respectively.

The findings in the present study suggest that the stimulating mechanism of PGE_1 in smooth muscle of guinea-pig stomach may mainly depend on a release of bound Ca in the cell and partly depend on a Ca influx from the extracellular origin, and ACh-induced contraction may also depend on 2 Ca origins, in which the influx Ca will be more available than in PGE_1.

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Examination of the potential mutagenicity of hair dye constituents using the micronucleus test[1]

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Summary. 12 compounds which are constituents of hair dyes or chemically related aromatic amines, aminophenols, their nitroderivatives and aromatic hydroxyderivatives were examined for evidence of mutagenic potential by means of the micronucleus test. None of the compounds tested caused an increase in the incidence of micronucleated erythrocytes after oral dosing.

There has been increasing concern that some constituents of hair dyes, mainly aromatic amines, aminophenols, their nitroderivatives and hydroxyderivatives may be mutagens and possible carcinogens. Although some epidemiological evidence is available[2–4], much of the concern is based on the results of in vitro studies using microorganisms[5,6], cultures of human lymphocytes[5], Chinese hamster cells[7] and a mouse cell line[8], and on tumorigenicity studies[9]. However, other studies[10–12] have failed to show long-term toxicity, carcinogenic or teratogenic effects. The percutaneous absorption of the compounds has been studied in animals[13] and man[14].

The experimental work reported here was designed to determine the effects of the compounds on the genetic material of mammalian somatic cells when administered orally at the maximum tolerated dose, by means of the micronucleus test developed by Schmid et al.[15–18].

Materials and methods. The compounds examined were: p-phenylenediamine, 4-methoxy-m-phenylenediamine, 4-nitro-o-phenylenediamine, 2-nitro-p-phenylenediamine, p-aminophenol, m-aminophenol, 2-amino-4-nitrophenol, toluene-2, 5-diaminesulphate, resorcinol, 4-chlororesorcinol, 4-amino-2-hydroxytoluene, 1-naphthol.

Rats of the CFY strain (Sprague-Dawley descendants) weighing between 130 and 160 g were obtained from Anglia Laboratory Animals, Alconbury, Cambs., U.K., acclimatized in the laboratory for 1 week and then randomly allocated into groups each including 5 males and

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