34 EFFECT OF SENSITIZATION ON MODULATION OF A23187-INDUCED CONTRACTION OF GUINEA PIG TRACHEA.

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The effect of pretreatment (30 min) with isoprenaline (ISO), PGE_2, dibutyryl cyclic AMP (dbcAMP) and aminophylline (AM) were examined on A23187 (5.7μM)-induced contractions of tracheal spirals of normal (N) guinea pigs and of guinea pigs sensitized (S) with ovalbumin. ISO inhibited A23187-induced contraction of N trachea dose-dependently. In contrast ISO had a biphasic effect on S trachea. Low concentrations (10^{-8}-10^{-7}M) enhanced contraction, whereas higher concentrations (10^{-6}-10^{-5}M) were inhibitory. Both the inhibitory and enhancing effects of ISO were blocked by propranolol (10^{-6}M). PGE_2 (10^{-7}-10^{-5}M) and dbcAMP (10^{-3}M) both enhanced A23187-induced contraction of S trachea but had no significant effect on N tissue. No significant differences ($P>0.05$) exist between N and S tissue for responses in the presence of PGE_2. A lower concentration of dbcAMP (10^{-4}M) slightly inhibited (30±10%) contraction. AM showed no differentiation between S and N trachea. A low concentration (10^{-4}M) slightly enhanced (20-40%) and a higher concentration (10^{-3}M) inhibited (70-90%) A23187-induced contractions. The results suggest that sensitization induces intrinsic changes in the ability of trachea to react to agents that increase intracellular levels of cAMP, particularly ISO and dbcAMP. Pools of cAMP are known to be involved both in secretion and in modulation of mediator release and sensitization may affect the differential availability of these pools.
(Supported by MRC and AHFMR).

35 INHIBITION OF CALCIUM IONOPHORE (A23187)-INDUCED HISTAMINE RELEASE BY A NOVEL ANTI-ALLERGIC AGENT, 4-(p-CHLOROBENZYL)-2-(HEXAHYDRO-1-METHYL-1H-AZEPINE-4yl)-1(2H)-PHTHALAZINONE HYDROCHLORIDE (AZELASTINE; A5610).

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Azelastine is a new anti-allergic drug with strong and long lasting antihistaminic properties (Pharmacologist 23, 149, 1981; Arzneim-Forsch. 31, 1196, 1981). Azelastine inhibits histamine release induced by concanavalin-A + phosphatidyl serine and compound 48/80 from rat peritoneal mast cells (Pharmacologist 23, 161, 1981). In the present study, the ability of azelastine to inhibit histamine release induced by calcium ionophore, A23187 (0.2 μM) from rat peritoneal mast cells was investigated and compared to ketotifen, disodium cromoglycate (DSCG), theophylline and diphenhydramine. The concentrations required for 50% inhibition of histamine release, i.e. IC_{50}(μM), were: azelastine, 5 ± 0.5; diphenhydramine, 52 ± 7, and ketotifen, 200 ± 20. DSCG and theophylline in the concentration range of 0.1 to 1000 μM failed to exert any significant inhibition of histamine release. The order of the inhibitory activity on A23187-induced histamine release was azelastine>diphenhydramine>ketotifen>DSCG = theophylline. These and previously published observations demonstrate that azelastine exerts a selective and strong effect on Ca^{++}-dependent step(s) in histamine secretion (exocytosis) common to antigen, con A + PS and A23187.

36 ENZYME IMMUNOASSAY FOR HONEY BEE VENOM SPECIFIC IgG4

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Honey bee venom (HBV)-specific IgG has been found to inhibit allergic reactions in beekeepers and HBV-sensitive patients on HBV immunotherapy (IT). HBV-specific IgG4 subclass has been suggested as a possible blocking antibody. We developed an enzyme-linked immunoassay for HBV-specific IgG4. HBV (Sigma) 100 ug/ml is incubated overnight in polyvinylchloride microtiter wells (Dynatech). After washing, test serum diluted 1:100 in wash buffer is added for 2 hours at 37°C. Following aspiration and washing of wells, swine antihuman IgG4 (Nordic) 1:1000 is added for 3 hours at 37°C, followed by rabbit anti-swine IgG-Peroxydase (Nordic) 1:10,000 for 1 hour. The reaction is then developed with o-tolidine, stopped by transfer, and read at 520 nm. Twelve HBV-sensitive subjects (positive history for sting, systemic reaction, skin test, and RAST) were studied before and while on HBV IT and compared to 11 beekeepers and 23 healthy controls. The table shows that HBV-sensitive subjects had higher HBV IgG4 levels before IT than controls, with a further increase on HBV IT to levels comparable to those in beekeepers.

Groups are as follows, with Mean OD_{520} ± SD : HBV-sensitive, on IT : 209±71 - HBV-sensitive, pre-IT : 140±85 ($p<.025$) - Controls : 84±16 ($p<.005$) - Beekeepers : 208±41 ($p<.0005$)

HBV-specific IgG4 thus appears at higher levels to be associated with protection. The micro-ELISA is a convenient, fast, and widely applicable method for detection of antigen-specific IgG4.