Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue4abst161P.pdf

Effects Of Selective Blockade Of EP_1, EP_2 And TP Receptors In Antigen-Induced Contractions In The Guinea-pig Trachea

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Histamine and cysteinyl-leukotrienes are established mediators responsible for antigen-induced bronchoconstriction in humans and guinea-pigs (GP). However, the experiments in isolated smooth muscle preparations have been made in the presence of inhibitors of the cyclo-oxygenase (COX) reaction that catalyse biosynthesis of prostaglandins (PG), leaving considerable uncertainty about the role of different COX products for the response to antigen.

The aim of this study was to characterise the role of COX metabolites in the antigen-induced contraction of the GP trachea using selective inhibitors and antagonists. Since constitutively released PGE_2 maintains a powerful tone in the GP trachea, the effect of other potential COX metabolites during the antigen-induced contraction were investigated comparing the effect of COX-inhibition with selective antagonism of EP_{1-2} receptors plus other PG receptors.

Male albino GP (Dunkin-Hartley;400-450g) were sensitized by single peritoneal injections containing 100µg ovalbumin (OVA) and 0.1g $Al(OH)_3$ and humanely killed 14 days later by sodium pentobarbital. Intact tracheal ring-segments were placed in tissue organ-baths containing Krebs-Ringer PSS at 37°C. Cumulative concentrations of OVA (0.1ng/mL to 0.1mg/mL) were administrated in the absence and presence inhibitors/antagonists which were given 45min prior. Pharmacological tools used were; COX-inhibitor (indomethacin;3µM) and 5-lipoxygenase-activating protein (FLAP)-inhibitor (MK-886;10µM) as well as receptor antagonists for EP_1 (ONO-8130;0.1µM), EP_2 (PF-04418948;10µM), TP (SQ-29,548;1µM), H_1 (mepyramine;1µM) and H_2 (metiamide;1µM). Isometrical force was measured compared to maximal contraction (histamine;1mM, acetylcholine;1mM and KCl;60mM) in relation to maximal relaxation (papaverine;0.1mM and sodium nitroprusside;0.1mM). Statistical analysis was performed using One-Way ANOVA.

In controls, challenge with OVA induced concentration-dependent contractions from a basal tone of 35% reaching 76%, compared to the maximal tissue response (Table 1). Both COX-inhibition and antagonism of the EP_1 receptors abolished the basal tone ($p<0.05$), however, the antigen-induced contraction was greater in amplitude in presence of COX inhibition ($p<0.05$) compared to EP_1 receptor antagonism ($p<0.05$). Inhibition of FLAP and H_{1-2} receptors had no significant effects on the antigen-induced contraction when given separately but a significant inhibition was observed when administered in combination in the presence of either COX inhibition or EP_1 antagonism ($p<0.05$). Moreover, the antigen-induced contraction was completely abolished when the TP receptor antagonist was added to FLAP inhibition and antihistamines after EP_1 antagonism ($p<0.05$). Irrespective of which drugs that were used to block other pathways, addition of the EP_2 receptor antagonist increased the antigen-induced contraction with 25% ($p<0.05$).

In conclusion, by selectively antagonising the EP_{1-2} receptors it was possible to unmask a substantial component of the antigen-induced contraction that was mediated by a mediator acting on the TP receptor. This component has not been possible to assess in previous experiments when the powerful effect of PGE_2 is unopposed.

Table 1: Initial tone and E_{max} in GP trachea in response to ovalbumin

Treatment	**Initial tone (% of max)**	**E_{max} (% of max)**
Control	35.4±4.9	75.9±2.4
I	0.6±3.8	87.1±3.9
O	2.7±2.6	57.7±1.6
IMk	1.8±4.0	89.7±2.7
IM	1.9±2.1	91.0±4.3
IMMk	0.1±2.3	38.0±2.9
OMMk	2.6±2.3	23.1±1.6
OSMMk	0	0
OP	1.9±0.3	83.8±2.8
OPMMk	0.1±4.4	47.6±3.4
OSPMMk	0.3±2.4	28.0±2.7

I=Indomethacin, O=ONO-8130, Mk=MK-886, M=mepyramine&metiamid, S=SQ-29,548 and P=PF-04418948. All data are presented as mean±SEM (n=8-23).