Effect of chronic pretreatment with sympathomimetic bronchodilator drugs on the responsiveness of guinea-pig lung adenylate cyclase *in vitro*

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Tolerance to the cardiac stimulatory and bronchodilator effect of the sympathomimetic drugs used in the treatment of asthma has been shown to develop in man and the guinea-pig respectively (Conolly, Davies, Dollery & George, 1971; Bouhuys, Douglas & Lewis, 1972; Benoy, Elfellah, Schneider & Wade, 1975). However, the mechanism(s) by which such tolerance is brought about is unknown. One possibility is that the drugs used can desensitize the β-adrenoceptors. Since there is strong evidence to support the hypothesis that β-adrenoceptors are associated with adenylate cyclase (Robison, Butcher & Sutherland, 1971), we have investigated the effect of pretreatment with sympathomimetic bronchodilator drugs on guinea-pig adenylate cyclase activity *in vitro*.

The animals were pretreated as previously described (Benoy *et al.*, 1975), killed by cervical dislocation and lung slices (1 mm in thickness) prepared using a McIlwain tissue chopper. In some experiments, tracheal rings were also used. Incubation conditions and methods were as described by Kakiuchi & Rall (1968) with the following modifications: the incubation was terminated by immersing the tubes in a boiling water bath for 3 min and homogenates of the tissue were prepared in the incubation medium using a Silverson homogenizer. One ml aliquots were removed for protein determination (Lowry, Rosebrough, Farr & Randall, 1951) and the remaining solution was centrifuged at 2000 *g* for 10 min and used for the assay of cyclic AMP using the method of Gilman (1970) as modified by Torey, Oldham & Whelan (1974).

There was no change in basal adenylate cyclase activity in either lung slices or tracheal rings after any drug pretreatment. However, after pretreatment of animals with isoprenaline for 7 days, but not for 3 days, with thrice daily s.c. injections of 5 μg/kg, the sensitivity of adenylate cyclase to isoprenaline (10^{-4}M) was reduced in lung slices [control 173 ± 25 (10); pretreated 86 ± 12 (10) ($P < 0.01$); results depict mean activity expressed as pmol cAMP.mg protein^{-1} 6 min^{-1} minus basal activity $\pm$s.e. mean] and also in tracheal rings [control 24 ± 6 (6); pretreated 8 ± 3 (6) ($P < 0.01$); results expressed as pmol cAMP tracheal ring^{-6} 6 min^{-1}]. In animals similarly pretreated with isoprenaline, cross-tolerance was shown in lung slices challenged with noradrenaline (5×10^{-5}M) [Control 130 ± 20 (4); pretreated 52 ± 8 (4) ($P < 0.02$)] but not when challenged with prostaglandin E_1 (5×10^{-6} M) [control 387 ± 28 (6); pretreated 340 ± 50 (6)].

When guinea-pigs were pretreated with salbutamol (thrice daily s.c. injections of 10 μg/kg) a cross-tolerance developed to isoprenaline, but not to prostaglandin E_1, in lung slices and tracheal rings.

Since we have previously found (Elfellah and Turnbull—to be published) that lung phosphodiesterase activity is unaffected by the above drug pretreatments, our results suggest that the reduced sensitivity of adenylate cyclase found in the present experiments may be due to a desensitization of the enzyme caused by the prior *in vivo* exposure to the bronchodilator drugs. It is tempting to speculate that this phenomenon may be related to the development of tolerance to bronchodilator drugs in asthmatic subjects.

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References

BENOY, C.T., ELFELLAH, M.S., SCHNEIDER, R. & WADE, O.L. (1975). Tolerance to sympathomimetic bronchodilators in guinea-pig isolated lungs following chronic administration *in vivo*. *Br. J. Pharmac.*, **55**, 547–555.

BOUHUYS, A., DOUGLAS, J.S. & LEWIS, A.J. (1972). Hypersensitivity to adrenoceptor agents in the guinea-pig *in vitro* and *in vivo*. *Br. J. Pharmac.*, **46**, 520–522P.

CONOLLY, M.E., DAVIES, D.S., DOLLERY, C.T. & GEORGE, C.F. (1971). Resistance to β-adrenoceptor stimulation (a possible explanation for the rise in asthma deaths). *Br. J. Pharmac.*, **43**, 389–402.

GILMAN, A. (1970). A protein binding assay for adenosine 3′,5′ cyclic monophosphate. *Proc. natn. Acad. Sci. U.S.A.*, **67**, 305–312.

KAKIUCHI, S. & RALL, T.W. (1968). The influence of chemical agents on the accumulation of adenosine 3′,5′ phosphate in slices of rabbit cerebellum. *Molec. Pharmac.*, **4**, 367–378.

LOWRY, D.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

ROBISON, G.A., BUTCHER, R.W. & SUTHERLAND, E.W. (1971). See *Cyclic AMP*. New York and London: Academic Press.

TOREY, K.C., OLDHAM, K.G. & WHELAN, J.A.A. (1974). A simple direct assay for cyclic AMP in plasma and other biological samples using an improved competitive protein binding technique. *Clinica chim. Acta*, **56**, 221–234.