

## Drug desensitization and cross-desensitization in guinea-pig ileum

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Drug desensitization was assessed in guinea-pig ileum strips suspended in Ringer solution employing isometric recording. An electronic compensating device (Schild & Seaford, 1970) automatically readjusted the length of the muscle thus ensuring a constant initial tension. Desensitizing drugs were administered for a period of 8-10 min in a high concentration (50-100 times that needed to establish a dose-response curve) and the degree of subsequent desensitization was expressed in terms of dose-ratio usually 10 min afterwards.

The main findings were as follows: 1. Degree of desensitization depended on concentration and period of contact with the desensitizing drug. 2. Strong cross-desensitization was seen after acetylcholine desensitization of histamine responses (ACh  $\rightarrow$  Hi); less strongly by Hi  $\rightarrow$  ACh: strong cross-desensitization by Hi  $\rightarrow$  5-HT; little or none by 5-HT  $\rightarrow$  Hi: strong cross-desensitization by Hi  $\rightarrow$  bradykinin (BK). 3. The degree of desensitization was related to the  $\text{Ca}^{++}$  content of the Ringer solution during the desensitization

period (using hepes Ringer, Good, Winget, Winter, Connolly, Izawa & Singh, 1966, which does not precipitate calcium, as the desensitizing solution). When the desensitizing solution contained 4 mM  $\text{Ca}^{++}$  in place of 0.2 mM  $\text{Ca}^{++}$ , duration and degree of both auto-desensitization Hi  $\rightarrow$  Hi (Schild, 1973), ACh  $\rightarrow$  ACh, BK  $\rightarrow$  BK and cross-desensitization ACh  $\rightarrow$  Hi, Hi  $\rightarrow$  BK were significantly diminished. 4. Desensitization Hi  $\rightarrow$  Hi was obtainable in the complete absence of calcium in solution suggesting that calcium is not required for the interaction of histamine with receptors.

These findings suggest that drug desensitization in guinea pig ileum may, depending on the drug used, be unspecific or drug-specific and that it is related to the calcium content of the desensitizing solution. It seems possible that the effect of calcium is exerted at a post-receptor level common to different drugs.

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## The effect of isoprenaline on $^{86}\text{Rb}$ uptake by horse lymphocytes *in vitro*

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Catecholamines affect a number of functions of leucocytes; these functions include inhibition of histamine release (Lichtenstein & Margolis, 1968), inhibition of immune cytolysis (Henney, Bourne & Lichtenstein, 1972) and inhibition of sheep red blood cell plaque formation (Melmon, Bourne, Weinstein, Shearer, Brauminger & Kraur, 1974).

The possible importance of changes in cation distribution during mitogen-induced transformation of lymphocytes (Quastel & Kaplan, 1970; Allwood, Asherson, Davey & Goodford, 1971) led us to carry out a study on the effect of

isoprenaline on the uptake of  $^{86}\text{Rb}$  by lymphocytes.

Lymphocytes were isolated from horse peripheral blood. To remove granulocytes, the lymphocyte-rich plasma, obtained by gravity sedimentation for 15 min, was passaged through three (7.0 x 1.4 cm) glass wool columns set up in series. The lymphocyte-rich effluent obtained from these columns was run onto a fourth set of (12.0 x 1.4 cm) glass wool columns which were incubated at 37°C for 1 hour. Elution of these latter columns with medium 199 (pH 7.4, gassed with 5%  $\text{CO}_2$  in air) followed by lysis of the remaining red cells with 155 mM  $\text{NH}_4\text{Cl}$  gave a fraction of lymphocytes (always greater than 97% pure from other cell types), which were suspended in medium 199 containing 20% heat-inactivated homologous serum at a concentration between  $2 \cdot 10 \times 10^6$  cells  $\text{ml}^{-1}$ .

The lymphocytes obtained by this method were maintained on a culture-tube roller, without

gassing, at 37°C and were allowed to recover for at least 1 hour. After this time they appeared morphologically normal under light and electron microscopy, excluded dyes such as trypan blue and eosin Y, showed an increased uptake of  $^{14}\text{C}$ -thymidine in the presence of  $20\text{ }\mu\text{g ml}^{-1}$  phytohaemagglutinin (PHA) and exhibited 50% inhibition of  $^{86}\text{Rb}$  uptake with  $10^{-5}\text{ M}$  ouabain.

Cells were incubated at 37°C for 15 min in the presence of  $^{86}\text{Rb}$  and were removed from the surrounding medium by spinning for 1 min in a microcentrifuge at 12,500 g. Correction for any remaining medium was calculated using  $^{14}\text{C}$ -sorbitol. When isoprenaline was administered at a range of concentrations ( $10^{-4}$ - $10^{-10}\text{ M}$ ) a significant ( $P < 0.01$ ) increase in  $^{86}\text{Rb}$  uptake over control was seen with a maximum at  $10^{-7}\text{ M}$ .

Isoprenaline has been shown to increase significantly the synthesis of IgG by human peripheral lymphocytes over the range  $10^{-7}$ - $10^{-10}\text{ M}$ , with a maximum at  $10^{-8}\text{ M}$  (Sherman, Smith & Middleton, 1973). Therefore it would seem that a peak activity of isoprenaline at  $10^{-7}\text{ M}$  for  $^{86}\text{Rb}$  uptake may not be unrelated to another isoprenaline effect on lymphocytes. However, the importance of the role of cations in catecholamine-stimulated cellular activity still has to be elucidated.

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## Stimulant effects of 5-hydroxytryptamine on cardiac sympathetic nerves

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5-Hydroxytryptamine (5-HT) stimulates the isolated rabbit heart and its effects are abolished by pretreatment with reserpine (Jacob & Poite-Bevierre, 1960) suggesting mediation by catecholamine release. This action has now been further investigated. In particular, 5-HT has been compared with noradrenaline, which acts directly on the receptors, with tyramine, which releases noradrenaline by stoichiometric displacement from the neurone (Trendelenburg, 1972) and with dimethylphenylpiperazinium (DMPP) which releases noradrenaline by depolarization resulting from activation of nicotinic receptors (Muscholl, 1970).

Hearts were removed from rabbits given heparin ( $500\text{ }\mu\text{g/kg}$ ) 5 min before killing and perfused at constant pressure by the Langendorff technique with Tyrode solution containing atropine ( $5 \times 10^{-7}\text{ g/ml}$ ) at 37°C. Right atrial and ventricular tensions and right ventricular rate were recorded as previously described (Fozard & Muscholl, 1971). Drugs were either given by bolus injection or incorporated into the perfusion fluid.

Noradrenaline ( $0.04$ - $40\text{ }\mu\text{g}$ ), 5-HT ( $0.5$ - $512\text{ }\mu\text{g}$ ), DMPP ( $5$ - $320\text{ }\mu\text{g}$ ) and tyramine ( $2.5$ - $640\text{ }\mu\text{g}$ ) caused dose-dependent increases in the rate and force of cardiac contraction. Propranolol reduced these responses at low concentrations. Using the rate response, the  $\text{pA}_2$  values obtained for the antagonism of noradrenaline, 5-HT, DMPP and tyramine by propranolol were  $8.42 \pm 0.08$ ,  $n = 8$ ;  $8.43 \pm 0.24$ ,  $n = 5$ ;  $8.45 \pm 0.16$ ,  $n = 3$ ;  $8.29 \pm 0.12$ ,  $n = 3$  respectively. Pretreatment of animals with 6-hydroxydopamine (Fozard, Kelly & Small, 1973) markedly reduced responses of the hearts to 5-HT, DMPP and tyramine despite an increase in the sensitivity to noradrenaline.

Tachyphylaxis developed rapidly to 5-HT and