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}C-thymidine in the presence of 20 μg ml^{-1} phytohaemagglutinin (PHA) and exhibited 50% inhibition of ^{86}Rb uptake with 10^{-5} M ouabain.

Cells were incubated at 37° C for 15 min in the presence of ^{86}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}C-sorbitol. When isoprenaline was administered at a range of concentrations (10^{-4}-10^{-10} M) a significant ($P < 0.01$) increase in ^{86}Rb uptake over control was seen with a maximum at 10^{-7} M.

Isoprenaline has been shown to increase significantly the synthesis of IgG by human peripheral lymphocytes over the range 10^{-7}-10^{-10} M, with a maximum at 10^{-8} M (Sherman, Smith & Middleton, 1973). Therefore it would seem that a peak activity of isoprenaline at 10^{-7} M for ^{86}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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References

ALLWOOD, G., ASHERSON, G.L., DAVEY, M.J. & GOODFORD, P.J. (1971). The early uptake of radioactive calcium by human lymphocytes treated with phytohaemagglutinin. *Immunology*, **21**, 509-516.

HENNEY, C.S., BOURNE, H.R. & LICHTENSTEIN, L.M. (1972). The role of cyclic 3' 5'-adenosine monophosphate in the specific cytolytic activity of lymphocytes. *J. Immunol.*, **108**, 1526-1534.

LICHTENSTEIN, L.M. & MARGOLIS, S. (1968). Histamine release *in vitro*: Inhibition by catecholamines and methylxanthines. *Science*, **161**, 902-903.

MELMON, K.L., BOURNE, H.R., WEINSTEIN, Y., SHEARER, G.M., BRAUMINGER, S. & KRAUR, J. (1974). Haemolytic plaque formation by leukocytes *in vitro:* control by vasoactive hormones. *J. Clin. Invest.*, **53**, 13-20.

QUASTEL, M.R. & KAPLAN, J.G. (1970). Early stimulation of potassium uptake in lymphocytes treated with PHA. *Expt. Cell Res.*, **63**, 230-233.

SHERMAN, N.A., SMITH, R.S. & MIDDLETON, E. (1973). Effect of adrenergic compounds, aminophylline and hydrocortisone, on *in vitro* immunoglobulin synthesis by normal human peripheral lymphocytes. *J. Allergy Clin. Immunol.* **52**, 13-22.

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 μ/kg) 5 min before killing and perfused at constant pressure by the Langendorff technique with Tyrode solution containing atropine (5×10^{-7} 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 μg), 5-HT (0.5-512 μg), DMPP (5-320 μg) and tyramine (2.5-640 μg) caused dose-dependent increases in the rate and force of cardiac contraction. Propranolol reduced these responses at low concentrations. Using the rate response, the pA_2 values obtained for the antagonism of noradrenaline, 5-HT, DMPP and tyramine by propranolol were 8.42 ± 0.08, n = 8; 8.43 ± 0.24, n = 5; 8.45 ± 0.16, n = 3; 8.29 ± 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

DMPP. During full tachyphylaxis to 5-HT normal responses to noradrenaline, DMPP and tyramine could be obtained. In contrast, with the hearts desensitized to DMPP, responses to 5-HT were also abolished, although those to noradrenaline and tyramine were little affected. Colchicine (10^{-4} to 10^{-3} g/ml) inhibited responses to 5-HT and DMPP concentration-dependently, but had no significant effects on responses to noradrenaline or tyramine.

After the cardiac noradrenaline stores were labelled by perfusion with ^{3}H-(−) noradrenaline (10 ng/ml–Starke, 1971), bolus injections of 5-HT (512 μg), tyramine (40 μg) and DMPP (40 μg) evoked tritium release from the hearts. The pattern of tritium appearance in the perfusate after 5-HT showed a peak, 10-20 s after the injection with little release being evident after 1 minute. A qualitatively identical pattern was obtained with DMPP. In contrast, tyramine released tritium at a constant rate during the 3 min period immediately following the injection. Reducing the Tyrode calcium ion concentration from 3.6-0.2 mEq/l did not affect the tritium release after tyramine, although the release evoked by 5-HT and DMPP was markedly inhibited.

The results confirm the suggestion that 5-HT stimulant responses on the rabbit heart are the result of noradrenaline release. They further suggest that the site of release is the terminal sympathetic nerve network. The mechanism of release shows more similarities to the DMPP release mechanism (depolarization) than to that of tyramine (neuronal uptake and stoichiometric displacement).

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References

FOZARD, J.R., KELLY, M.J. & SMALL, R.C. (1973). Chemical sympathectomy of the rabbit with 6-hydroxydopamine. *Br. J. Pharmac.*, **49**, 182P-183P.

FOZARD, J.R. & MUSCHOLL, E. (1971). A useful muscarinic parameter and the differential recording of atrial and ventricular tension in the perfused rabbit heart. *Naunyn-Schmiedebergs Arch. Pharmak.*, **270**, 319-325.

JACOB, J. & POITE-BEVIERRE, M. (1960). Action de la Sérotonine et de la benzyl-1-dimethyl 2,5-sérotonine sur le coeur isolé de lapin. *Archs int. Pharmacodyn. Ther.*, **127**, 11-16.

MUSCHOLL, E. (1970). Cholinomimetic drugs and the adrenergic transmitter. In: *New aspects on storage and release mechanisms of catecholamines*, pp. 168-186, Berlin and Heidelberg: Springer-Verlag.

STARKE, K. (1971). Action of angiotensin on uptake release and metabolism of ^{14}C-noradrenaline by isolated rabbit hearts. *Eur. J. Pharmac.*, **14**, 112-123.

TRENDELENBURG, U. (1972). Classification of sympathomimetic amines. In: *Catecholamines, Handb. exp. Pharmac. N.S.*, vol. 33, Ed. Blaschko, H. & Muscholl, E. pp. 336-362. Berlin and Heidelberg: Springer-Verlag.

5-Hydroxytryptamine synthesis in the isolated perfused rat brain

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Isolated perfused organs provide an experimental system for the study of metabolism in an organ retaining its structural integrity. Little work has been done upon monoamine metabolism in the perfused brain. We have developed a method for the *in situ* perfusion of rat brain which excludes the influence of extracranial tissues on tryptophan metabolism and this paper describes the use of this method in the study of 5-hydroxytryptamine metabolism.

Rat brains were perfused using the method described by Woods, Graham & Grahame-Smith (1974). The metabolic, and histological properties of this preparation are very similar to those observed *in vivo*. For example, the rate of glucose uptake was 0.79 μmol min^{-1} gram^{-1} in the presence of 5 mmol/l glucose, and the rate of acetoacetate uptake was concentration dependent being 0.14 μmol min^{-1} gram^{-1} after loading with 1 mmol/l acetoacetate and 0.24 μmol/min^{-1} gram^{-1} with 2 mmol/l.

For the study of 5-HT synthesis rats were anaesthetized with Nembutal (60 mg/kg i.p.) and the brains perfused with a medium containing glucose (10 mmol/l) together with tranylcypromine (1 mmol/l) and tryptophan (0.1 or 1.0 mmol/l). After perfusion for varying times the brains were rapidly removed from the skull and stored at −20° C before determination of 5-HT and tryptophan concentrations.

Anaesthesia and preparation of the brain for perfusion resulted in a small increase in brain 5-HT concentration when compared with brains obtained after cervical dislocation (from 0.46 to 0.53 μg/g).