preparation to show a pressor response is not solely a result of abolishing the centrally mediated effects of angiotensin II.

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Intracellular enzymes in local lymph during homograft rejection

M. K. JASANI and G. P. LEWIS*
CIBA Laboratories, Horsham, Sussex

The concentrations of some intracellular enzymes as well as that of protein increases in the lymph draining a rabbit hind limb after the limb has been subjected to thermal or chemical injury, but the nature of the enzyme pattern depends upon the degree of cellular injury (Lewis, 1969; Boyles, Lewis & Westcott, 1970). In the present experiments a different kind of injury has been investigated in a similar way—the immunological response to skin homotransplantation.

Rabbits were anaesthetized with pentobarbitone sodium (40 mg/kg) and a cannula inserted into the main femoral lymphatic of the right hind limb as described by Lewis (1969). The operation was carried out under aseptic conditions and the rabbit allowed to recover. Six to eight full thickness skin grafts were made on the right hind limb between the knee and ankle. For autografts the grafts were transplanted to the opposite side of the right limb of the same animal; for homografts they were exchanged between Norfolk and New Zealand White rabbits.

In the supernatant of the lymph obtained after centrifugation, the activities of four of the six enzymes examined—cathepsin, acid phosphatase, glutamic pyruvic transaminase (GPT), and glutamic oxalacetic transaminase (GOP) increased during the first 5 days after the grafting of autografts or homografts. These increases probably result from the non-specific injury of transplantation. With autografts the increases then subsided, but with homografts they increased further during, and immediately after, rejection when there was an even greater increase in the activities of the other two enzymes examined—LDH and β-glucuronidase. In the cell pellets obtained after centrifugation of the lymph the activities of the six enzymes did not increase during the first 5 days although subsequently they all increased with homografts but not with autografts. Since there were no concomitant increases in the lymphocyte counts, some of the lymphocytes must have become activated during the time leading up to rejection in order to contain higher enzyme activities. It has already been shown that a lymphatic connexion between the graft itself and the host tissue is not a prerequisite of homograft rejection and that such a connexion is not usually established under the present experimental conditions (Jasani & Lewis, 1970). It is concluded, therefore, that the increases in enzyme activities of the lymph collected during and after rejection result from 'activated lymphocytes' which infiltrate the graft bed and functional tissue and subsequently undergo necrosis.

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Autonomic receptors and choline uptake in embryonic chick myocardial cell cultures

D. E. CLARKE*, R. J. ERTEL, F. R. FRANKE and G. OUYANG

Department of Pharmacology, School of Pharmacy, University of Pittsburgh, and Department of Cardiovascular Diseases, The Western Pennsylvania Hospital, Pittsburgh, Pennsylvania 15213, USA

Autonomic receptors are believed to be present in whole chick embryonic hearts (McCarty, Lee & Shideman, 1960; Roberts, Gimeno & Webb, 1965; Coraboeuf, Obrecht-Coutris & Le Douarin, 1970) but no quantitative study to elucidate autonomic receptor mechanisms in culture cells has been conducted. The following experiments have been carried out in an attempt to identify and characterize such mechanisms. In addition, choline accumulation by the cultured cells has been investigated since Coraboeuf, Le Douarin & Obrecht-Coutris (1970) have recently demonstrated the release of acetylcholine from non-innervated (3-day-old) chick embryonic hearts.

Experiments were performed on spontaneously contracting cultured chick myocardial cells at 37°C, prepared from 7-day-old whole embryonic hearts. The cells were viewed using an inverted phase microscope and drug-induced changes in rate were recorded.

Typical β-adrenoceptors were identified using various catecholamine agonists and antagonists. For instance, the rate-increasing action of isoprenaline (0·001–0·2 μg/ml) was blocked by propranolol (0·01–0·04 μg/ml) but not by phentolamine (1 μg/ml) or hyoscine (1 μg/ml). Tyramine (10 and 25 μg/ml), nicotine (0·1–10 μg/ml) and bretylium (100 μg/ml) failed to alter rate, indicating the possible absence of endogenous, intracellular catecholamines.

A muscarinic-like receptor appears to be present in cultured cells, although it seems atypical with respect to certain drug interactions. Thus the intensity of effects to acetylcholine (0·1–10 μg/ml) was not related to concentration, and an initial rate-increasing effect, followed by a subsequent slowing, was often observed. Although all effects of acetylcholine were blocked by scopolamine (1·0 μg/ml), but not by nicotinic receptor blocking agents, they were also antagonized by physostigmine (2 μg/ml) and neostigmine (1 μg/ml). In higher concentrations these agents themselves produced a concentration-dependent increase in rate whereas di-isopropylfluorophosphonate (0·01–0·5 μg/ml) progressively decreased rate but failed to antagonize the rate-increasing action of acetylcholine.

^{14}C-Choline, added to the cultures, exhibited a time-related accumulation into the myocardial cells which was antagonized by hemicholinium-3 in a concentration-dependent manner and was completely blocked at 4°C. The possible non-neuronal synthesis of acetylcholine in chick myocardial cells is under investigation, since this substance has long been suspected of playing an important role in myocardial cell rhythmicity.