REFERENCES

BOYLES, SUSAN, LEWIS, G. P. & WESTCOTT, BARBARA (1970). Changes in the composition of hind limb lymph after chemical injury. *Br. J. Pharmac.*, **39**, 798–808.

JASANI, M. K. & LEWIS, G. P. (1970). Lymph flow and biochemical composition during homograft rejection. *J. Physiol., Lond.*, **207**, 70–71*P*.

LEWIS, G. P. (1969). Changes in the composition of rabbit hind limb lymph after thermal injury. *J. Physiol., Lond.*, **205**, 619–634.

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.

REFERENCES

CORABOEUF, E., LE DOUARIN, G. & OBRECHT-COUTRIS, G. (1970). Release of acetylcholine by chick embryo heart before innervation. *J. Physiol., Lond.*, **206**, 383–395.

CORABOEUF, E., OBRECHT-COUTRIS, G. & LE DOUARIN, G. (1970). Acetylcholine and the embryonic heart. *Am. J. Cardiol.*, **25**, 285–291.

MCCARTY, L. P., LEE, W. C. & SHIDEMAN, F. E. (1960). Measurement of the ionotropic effects of drugs on the innervated and noninnervated embryonic chick heart. *J. Pharmac. exp. Ther.*, **129**, 315–321.

ROBERTS, C. M., GIMENO, M. A. & WEBB, J. L. (1965). On the role of acetylcholine in regulating the rate of the early chick embryo heart. *J. cell. comp. Physiol.*, **66**, 267–272.

Influence of calcium on responses of human isolated uterine tissue to fenfluramine

MARILYN J. KIRBY (introduced by P. TURNER)

Clinical Pharmacology Division, Medical Professorial Unit, St. Bartholomew's Hospital, London EC1

The actions of fenfluramine on isolated human vein and gut have been described (Coupar, Hedges, Metcalfe & Turner, 1969). This communication describes its effects on human isolated uterine tissue, obtained at hysterectomy, and placed in cold Krebs-

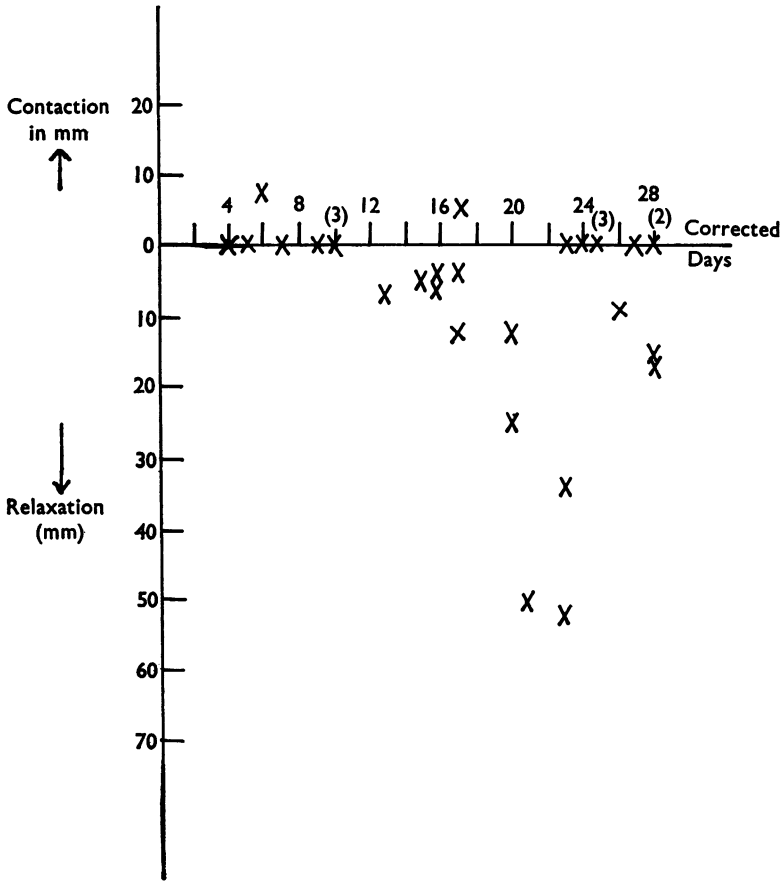

FIG. 1. Variations in response to fenfluramine on isolated strips of human uterine muscle, in 1·25 mM calcium Krebs solution, plotted against corrected day of the menstrual cycle.