Short communications

Biosynthesis of noradrenaline in organ cultured hearts

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In hearts of newborn mice, maintained in organ culture in an *in vitro* environment, noradrenaline levels were reduced after 24 h but subsequently recovered, reaching at three days values significantly greater than those found at birth. The increased synthesis of noradrenaline was blocked by the inhibitor of tyrosine hydroxylase, α-methyl tyrosine methyl ester. The site and control of noradrenaline biosynthesis in cultured newborn hearts are discussed.

Foetal mouse hearts (19–21 day term) maintained in organ culture have been reported to retain dose-related responsiveness to noradrenaline and adrenaline, but not to tyramine throughout the period of survival (Wildenthal, 1970). The loss of tyramine-induced sympathetic action on the heart after two days of culture was attributed to depletion of noradrenaline stores in the denervated, cultured heart (Wildenthal, 1970). The present experiments were designed, therefore, to ascertain whether the concentration of endogenous noradrenaline is altered in organ cultured hearts. We considered that such studies might also be of value if endogenous neurotransmitter stores are involved in the regulation of functional activity in transplanted cardiac tissue.

Methods.—Whole hearts, carefully isolated from newborn mice (1–6 h old), were cultured in medium 199 (Burroughs, Wellcome) which contained colostrum-free calf serum (32%) and glucose (5 mM). The hearts were cultured according to the method described by Wildenthal (1971) for mouse foetal hearts. Survival of the hearts was not assessed visually as in the studies of Wildenthal (1971) and Hughes & Longmore (1972), but in order to exclude observer error and bias, it was determined electrically. The presence of action potentials was used as the index of survival of the cultured hearts.

Estimation of noradrenaline was carried out on both freshly removed and surviving organ cultured hearts, by a radiometric assay (Saelens, Schoen & Kovascsics, 1967). The estimation was based on the finding of Axelrod (1962) that in the presence of phenyl ethanolamine-*N*-methyl transferase (PNMT), the tritiated methyl group of *S*-adenosyl-L-methionine ([^{3}H]SAM) is transferred to the primary amine nitrogen, resulting in the conversion of noradrenaline to adrenaline. Measurements were carried out on 4–6 hearts which were rinsed, pooled, weighed and homogenized in ice-cold 0·1 M sodium phosphate buffer solution (pH 5·0, containing 1 mM pargyline, 1 mM pyrogallol and 1 mM disodium edetate). The homogenate was acidified with cold perchloric acid (0·2 N, v/v 1:5), centrifuged (2 min, 1,000 *g*) and the supernatant neutralized with saturated potassium carbonate. The neutralized supernatant (pH 7·3) was incubated at 29° C with a standard enzyme-tritiated adenosyl mixture (acidified SAM chloride was added to [^{3}H] SAM, v/v 2:1, 20 μl of the [^{3}H] SAM mixture was added to 200 μl of PNMT). After 40 min the reaction was terminated and the incubate centrifuged at 35,000 *g*. Each supernatant was applied in triplicate to chromatography paper (Whatman No. P. 81) over an area previously spotted with cold carrier adrenaline. Noradrenaline standards and blanks were treated in the same manner as the tissue samples. The papers were chromatographed in a solvent system consisting of ammonium acetate (0·2 M) in glacial acetic acid (1·0 N) and isopropanol (v/v 2:1). The tritiated adrenaline was located by a combination of iodine vapour staining and ultra-violet fluorescence, eluted and measured in a Packard Tri-Carb liquid scintillation counter. Activity was expressed as μg noradrenaline per gram wet weight of heart tissue.

Results.—Noradrenaline levels in mouse hearts measured within 1–6 h of birth equalled $0{\cdot}1 \pm 0\,01$ μg/g tissue. This value was about five times lower than that obtained in adult mice ($0{\cdot}57 \pm 0{\cdot}13$ μg/g tissue). When hearts from newborn mice were cultured, they showed after 24 h a predictable fall in noradrenaline levels ($0{\cdot}065 \pm 0\,01$ μg/g tissue; Figure 1). But, with continued culture the concentration of noradrenaline in surviving hearts increased markedly and attained a value of $0{\cdot}36 \pm 0{\cdot}15$ μg/g tissue after three days of culture. The stimulation of synthesis was maximal at three days but declined thereafter.

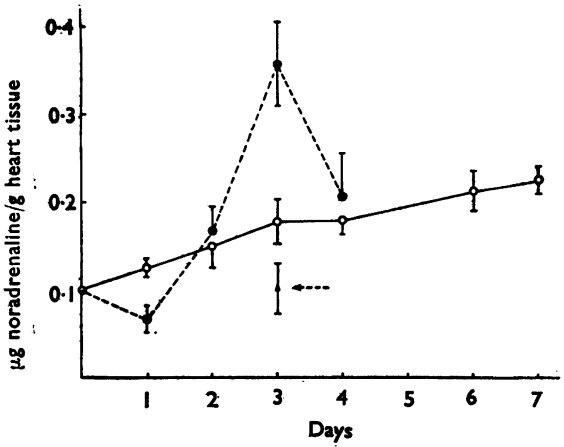

FIG. 1. Noradrenaline content of mice hearts. ○——○ values for uncultured hearts of 1–7 day old mice. ●- - -● values obtained for hearts removed from mice soon after birth (1–6 h) and cultured for 1–4 days. ◀- - - indicates noradrenaline levels in cultured hearts following addition of α-methyl tyrosine methyl ester (10 µM) to the culture medium after one day of culture; measurements carried out two days after addition of the tyrosine hydroxylase inhibitor. For each determination 4–10 hearts were pooled and estimations made in triplicate; each value represents the mean of 3–4 separate experiments ±S.E. of mean.

The conclusion that the rise in noradrenaline was due to increased biosynthesis was substantiated in experiments in which α-methyl tyrosine methyl ester (10 µM), an inhibitor of tyrosine hydroxylase (Moore & Dominic, 1966), was added to the bathing medium of hearts cultured successfully for 24 hours. The synthesis of noradrenaline was mainly blocked and the concentration of noradrenaline in the 3 day cultured hearts fell significantly ($0{\cdot}10 \pm 0{\cdot}04$ µg/g tissue). The question whether the increased levels of noradrenaline could be explained by a progressive fall in the weight of the cultured hearts was examined. After a slight reduction the weights of the cultured hearts remained relatively constant. The initial change in weight was small and not of sufficient magnitude to account for the increased noradrenaline values.

Discussion.—At birth (1–6 h old) the concentration of noradrenaline in the heart was low but subsequently increased showing a close correlation with age (see Figure 1). Sympathetic innervation of the heart at birth is considered to be less extensive than in the adult (Glowinski, Axelrod, Kopin & Wurtmann, 1964). Such a difference in adrenergic terminal numbers could account for the low values of noradrenaline obtained at birth. However, Wildenthal (1970) has reported that foetal mouse hearts (19–21 days old, full term) when maintained in organ culture retain responsiveness to noradrenaline and adrenaline but not to tyramine throughout the period of culture. The loss of response to tyramine was attributed to a depletion of noradrenaline stores. On the contrary, in the present study we have found that after an initial fall following the first 24 h of culture, the concentration of noradrenaline in the cultured hearts was significantly greater than that observed at birth and in the hearts of 3 day old mice (see Figure 1). In fact, the values in the 3 day cultured hearts equalled those observed in 4–6 week old mice. The inhibition by α-methyl tyrosine methyl ester confirmed the view that the increase in noradrenaline reflected increased biosynthesis. Experiments are in progress to determine the effect of organ culture on the activity of tyrosine hydroxylase and dopamine-β-hydroxylase in the hearts of newborn mice.

Sympathetic nerve terminals contain all the enzymes involved in the biosynthesis of noradrenaline (Axelrod, 1971) and in the isolated perfused heart the terminals are able to sustain rapid rates of noradrenaline synthesis (Levitt, Spector, Sjoerdsma & Udenfriend, 1965). With 10 mM tyrosine as substrate 0·2 µg noradrenaline/g guinea-pig heart tissue is synthesized in an hour and such a maximal

rate of noradrenaline synthesis gives a turnover rate of about 10% per hour. The concentration of tyrosine in the culture medium used in our experiments was 22 mM which is 100 times greater than the concentration of free tyrosine in tissues. The considerable excess of substrate which was available in the culture medium could easily sustain synthesis of noradrenaline.

Section of adrenergic nerves, removal of the right stellate ganglion (Glowinski *et al.*, 1964), or immunosympathectomy of the adult heart (Iversen, Glowinski & Axelrod, 1966) results in reduction of cardiac noradrenaline to very low levels or its disappearance. Our failure to record depletion of noradrenaline in hearts cultured for more than 24 h and up to 4 days suggests that perhaps the adrenergic nerve terminal in the newborn mouse heart is different from that in the adult. Such an interpretation is supported by the finding that the uptake, storage and metabolism of noradrenaline by the heart during the first week or two of life differs from that in the adult (Glowinski *et al.*, 1964; Iversen, 1967). Alternatively it may be considered that the heart tissue of newborn mice contains a high density of chromaffin cells (see Jacobowitz, 1967) with a latent capacity to synthesize noradrenaline, which is induced during culture.

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