Br. J. Pharmac. (1980), **70**, 515–517

DECREASED CONCENTRATION OF MYOCARDIAL α-ADRENOCEPTORS WITH INCREASING AGE IN FOETAL LAMBS

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Using [^{3}H]-dihydroergocryptine, we identified myocardial α-adrenoceptor binding sites in foetal lambs and demonstrated that the concentration of receptors decreased with increasing foetal age. The presence of the receptor in the foetus correlated with the presence of myocardial α-adrenergic responsiveness. However, we found neither the α-receptor binding site nor responsiveness to α-adrenoceptor stimulation in the myocardium of adult sheep.

Introduction Since Wenzel & Su (1966) first found that activation of myocardial α-adrenoceptors produced a contractile response, the existence and function of this receptor have been studied in the heart of many adult mammals (Schümann, 1980). However, we have found only two papers dealing with myocardial α-receptors in the foetus. Vapaavouri, Shinebourne, Williams, Heymann & Rudolph (1973), using chronically instrumented foetal lambs, demonstrated that phentolamine affected the foetal cardiovascular response earlier in gestation than did propranolol, suggesting the presence of functional α-receptors early in gestation in this species. Friedman (1973) found that the *in vitro* contractile response of sheep myocardium to noradrenaline, a mixed α- and β-agonist, was greater in the foetus than in the adult, although the response to isoprenaline, a β-agonist, was identical in the two preparations, implying a greater concentration of α-receptors in the foetus than in the adult.

To determine directly the development of α-receptors in the myocardium of foetal lambs, we assayed the binding of [^{3}H]-dihydroergocryptine, an α-antagonist, and compared the concentration of receptor binding sites in foetuses and their mothers (adults with a similar hormonal environment).

Methods Hearts were obtained from 14 foetal lambs at 114 to 147 days of gestation (term = 150 days) and from their mothers, who had been anaesthetized with pentobarbitone (60 to 120 mg/kg, i.v.).

Heart particulate preparation and binding assay We dissected the atria and coronary vessels from the ventricles, prepared ventricular particulates according to the method described by Sharma & Banerjee (1978), and determined the protein concentration by the method of Bradford (1976).

We performed a [^{3}H]-dihydroergocryptine (DHE) binding assay in a 250 μl volume containing the ventricular particulate preparation (protein concentration at 1 to 2 mg/ml), 50 mM Tris–HCl buffer (pH 7.4), 1 mM ascorbic acid, DHE (specific activity, 39 Ci/mmol, New England Nuclear) with 2% ethanol and with or without adrenoceptor agents. After incubating this solution at 30°C for 15 min, we added 5 ml of the buffer (4°C) and immediately filtered the sample through Whatman GF/C glass fibre filters to separate free and bound DHE. We then washed the filters with 20 ml of the buffer (4°C), dried the filters, and counted the radioactivity.

Two percent ethanol, which was added to increase the solubility of DHE, did not affect DHE binding. Specific binding, defined as binding in the absence of phentolamine minus binding in the presence of 10 μM phentolamine, was 30 to 70% of total binding in ventricular particulates from foetal lambs.

In the same particulate preparation, we measured the binding of [^{3}H]-dihydroalprenolol (DHA) (specific activity, 47 Ci/mmol, New England Nuclear), a β-antagonist. The conditions of the DHA binding assay were identical to those described for DHE. Binding in the presence of 10 μM (−)-alprenolol was defined as non-specific binding.

The inhibitory constants (K_i) for adrenoceptor agents were calculated from the equation $K_i = I_{50}/[1 + (L)/K_d]$ (Cheng & Prusoff, 1973).

Isometric contraction Ventricular muscle strips for isometric contraction were prepared according to the method of Shibata, Seriguchi, Iwadare, Ischida & Shibata (1980).

Results *[^{3}H]-dihydroergocryptine and [^{3}H]-dihydroalprenolol binding* In the foetal heart preparation, DHE binding was rapid, saturable (12 to 48 fmol/mg protein), of high affinity (dissociation constant = 1.2

0007-1188/80/120515-03 $01.00

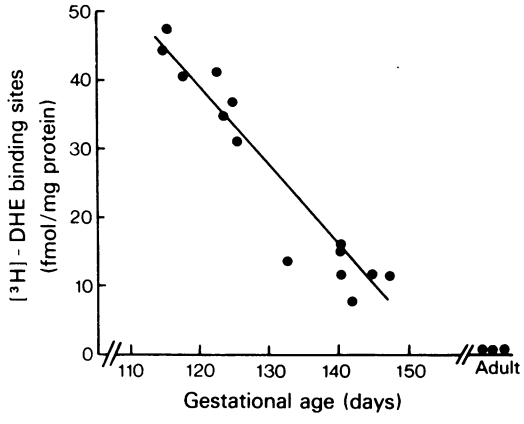

Figure 1 Concentration of [^{3}H]-dihydroergocryptine binding sites in hearts of foetal and adult sheep.

$\pm$ 0.2 nM, n = 14, mean $\pm$ s.e.), and reversible ($T_{\frac{1}{2}}$ = 4.6 min). Scatchard analysis of the data in equilibrium experiments ([DHE] = 0.3 to 9 nM) resulted in a line, indicating the presence of a single population of binding sites. Noradrenaline competed for this site stereoselectively, and the order of potency of agonists competing for DHE binding was (−)-noradrenaline (K_i = 2.5 $\pm$ 1.4 μM) $\simeq$ (−)-adrenaline (4.6 $\pm$ 2.0 μM) $\gg$ (−)-isoprenaline (>29 μM) (n = 3–4, each). This order is typical of interactions at the α-adrenoceptor (Ahlquist, 1948).

As shown in Figure 1, the number of myocardial α-receptor binding sites per mg of protein decreased with increasing foetal age (correlation coefficient r = −0.96, $P < 0.001$, n = 14). Similarly, the receptor concentration decreased with advancing foetal age when the data were expressed per g of ventricular wet weight (r = −0.68, $P < 0.01$). However, the DHE dissociation constant did not change during gestation (r = −0.48, $P > 0.1$).

We did not find high affinity DHE binding sites in heart particulates prepared from 3 adult sheep.

In the heart particulate preparation, we characterized DHA binding as consistent with β-adrenoceptor interactions (Cheng, Cornett, Kisloff, Goldfien & Roberts, 1979), and found that the concentration of β-receptors expressed per mg of protein or per g of tissue weight did not decrease significantly with increasing foetal age (r = 0.52 and 0.10, respectively; $P > 0.1$, n = 10). Although the concentration in ventricular preparations from adult sheep (138 $\pm$ 8 fmol/mg protein, n = 3) was slightly higher than that in foetal lambs (near term) (101 $\pm$ 7 fmol/mg protein, n = 4), the difference was not significant when the data were expressed per g of tissue weight (3.2 $\pm$ 0.3 pmol/g vs. 4.0 $\pm$ 0.4 pmol/g) ($P > 0.1$, Student's t-test).

Contractile responses to isoprenaline and phenylephrine
The concentration of isoprenaline required to cause a contractile response was 21.7 $\pm$ 14.2 nM (n = 3) in ventricular muscles of near term foetuses and 5.3 $\pm$ 2.6 nM (n = 3) in the adult. The difference was not significant ($P > 0.1$). However, in the presence of (−)-propranolol (0.5 μM), phenylephrine, an α-agonist, elicited a contractile response in the foetus (the threshold dose = 0.7 $\pm$ 0.3 μM) but not in the adult (n = 3, each). We could not study the *in vitro* response of ventricular strip preparations from young foetuses (<125 days gestational age) because of fragility of the tissue.

Discussion Using DHE, we demonstrated the presence of myocardial α-receptors in foetal lambs but not in adult sheep. This absence of α-receptors in adult sheep correlates with their lack of contractile response to α-adrenoceptor stimulation.

The decreased concentration of the α-receptor in the near term foetuses could have been due to a differential loss of plasma membrane during preparation. However, we used a relatively crude particulate fraction to minimize this possibility and also found that DHA binding did not decrease.

Since autonomic innervation increases with foetal age (Lebowitz, Novick & Rudolph, 1972), the higher concentration of α-receptors in the younger foetus cannot be a result of an increase in concentration of neural or presynaptic DHE binding sites. In a preliminary report, Felder, Eisner & Jose (1980) have recently found that α-receptors identified by [^{3}H]-WB-4101 (2-[2,6-dimethoxyphenoxyethyl]aminomethyl-1,4-benzodioxane) are present in hearts of young dogs (5 to 19 days) and the concentration decreases with increasing age, a finding consistent with our data. The presence of a relatively high concentration of α-receptors in foetal lambs at an earlier gestational age accompanying an unchanging concentration of β-receptors identified by DHA, may indicate a more prominent role for α-receptors in regulating myocardial contractility in early gestation.

This work was supported by U.S. NIH grant HL 24056. J.M.R. is the recipient of NIH Research Career Development Award (HD 00267). L.E.C. is the recipient of NIH Postdoctoral Fellowship (HD 05793).

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(Received August 19, 1980.)