Br. J. Pharmac. (1982), **75**, 479–483

β_2-ADRENOCEPTORS REGULATE INDUCTION OF MYOCARDIAL ORNITHINE DECARBOXYLASE IN MICE *in vivo*

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1 The pharmacological characteristics of the myocardial adrenoceptor of the mouse have been examined during embryogenesis by measuring ornithine decarboxylase (ODC, EC 4.1.1.17) induction.

2 A four fold elevation of ODC activity was observed after isoprenaline (10 mg/kg, s.c.), and enzyme activity was increased two to three fold following adrenaline (1 mg/kg, s.c.) or terbutaline given by direct injection to the foetus (10 μg/500 mg).

3 Pretreatment with the β-adrenoceptor antagonist, propranolol (10 mg/kg), totally blocked the increase in ODC activity.

4 Elevation of myocardial ODC activity was not inhibited by metoprolol, a relatively specific β_1-adrenoceptor antagonist, at a dose of 10 mg/kg.

5 Since the increase in ODC activity was blocked by a β-adrenoceptor antagonist (propranolol) and enzyme activity was stimulated by terbutaline, a β_2-agonist, we conclude that β_2-adrenoceptors are selectively coupled to the regulation of murine cardiac ODC activity following catecholamine stimulation.

Introduction

The ventricular myocardium is known to contain α- (Rabinowitz, Chuck, Kligerman & Parmley, 1975) and β-adrenoceptors with a ratio of β_1 to β_2 ca. 4:1 in rat heart (Minneman, Hegstrand & Molinoff, 1979). The foetal murine heart responds to catecholamine stimulation with an increase in the activity of the inducible enzyme, ornithine decarboxylase (ODC, EC 4.1.1.17) (Haddox, Womble, Larson, Roeske & Russell, 1981). Induction of ODC, the rate-limiting enzyme in polyamine biosynthesis, is a ubiquitous component of the growth response (Cohen, 1971; Russell, 1980). Changes in cellular polyamine concentrations have been shown to parallel RNA and protein synthetic rates during embryogenesis and in hypertrophy and hyperplasia of adult tissues (Cohen, 1971; Russell & Durie, 1978).

Trophic hormones, drugs, mitogens and cyclic adenosine 3′,5′-monophosphate (cyclic AMP) analogues rapidly induce ODC in physiological growth systems (Russell, 1980) and the ODC stimulation has been demonstrated to occur in all tissues and organs tested to date. Consequently, the induction of ODC can be used as a rapid, specific index of increased RNA and protein synthesis.

Catecholamines have been repeatedly implicated in cardiac hypertrophy in studies of exogenously administered isoprenaline, adrenaline or noradrenaline (Fisher, Horst & Kopin, 1965; Nair, Cutilletta & Rabinowitz, 1968; Stanton, Brenner & Mayfield, 1969; Feldman & Russell, 1972; Laks, Morady & Swan, 1973; Byus, Chubb, Huxtable & Russell, 1976). Three fold elevations of the endogenous plasma catecholamine, adrenaline, have been shown to parallel cardiac hypertrophy in the dog (Womble, Haddox & Russell, 1978) whereas ablation of plasma adrenaline by adrenal medulla denervation prevents cardiac hypertrophy after aortic constriction (Womble, Larson, Copeland, Brown, Haddox & Russell, 1980). Since catecholamines possessing both α and β agonistic properties increase cyclic AMP and ODC in the heart (Keely, Corbin & Park, 1975; Warnica, Antony, Gibson & Harris, 1975; Byus *et al.*, 1976; Bareis & Slotkin, 1978; Fuller & Hemrick, 1978; Keely, Lincoln & Corbin, 1978), the present study examined the specific nature of the receptor(s) coupled to the ODC response in foetal murine heart by evaluation with both α and β agonists and antagonists.

0007-1188/82/030479-05 $01.00

Methods

Experiments were conducted on CD-1 (Charles River) foetal mouse hearts at 18 days of embryogenesis. The mothers were injected intraperitoneally, with the exception of adrenaline (subcutaneously) and terbutaline (intramuscularly in the hip area of individual foetuses). Terbutaline fails to cross the placenta (Bodin, Hansson, Ramsay & Ryrfeldt, 1972) and therefore individual foetal injections were required. Adrenoceptor antagonists were administered 20 min prior to agonists. Details of foetal injection techniques have been given previously (Haddox *et al.*, 1981). Four h after drug administration, the mothers were killed by cervical dislocation and the foetal hearts were rapidly excised and frozen by immersion in liquid nitrogen. Heart samples were stored at −80°C until assayed. ODC activity was determined by the method of Russell & Snyder (1968). Briefly, frozen tissue (approximately 15 mg) was homogenized with a Tissumizer (Tekmar) at 4°C in 1 ml of 50 mM $NaHPO_4$-$KHPO_4$, pH 7.2, containing 5 mM NaF, 0.1 mM disodium edetate (EDTA), 2 mM dithiothreitol and 0.06 mM pyridoxal phosphate. The homogenates were centrifuged at 10,000 *g* for 5 min and two 200 μl aliquots of the resulting supernatant assayed for ODC activity. Incubations were conducted at 37°C for 60 min. The ODC enzymatic reaction was initiated by the addition of 0.2 μCi L-[^{14}C]-ornithine (50 mCi/mmol) (Amersham) and unlabelled ornithine as substrate to a final concentration of 0.25 mM. The reaction was terminated by the addition of 0.2 ml of 1 M citric acid after 60 min. The assay tubes were allowed to equilibrate for another 15 min and the $^{14}CO_2$ evolved was trapped by 20 μl of 2 N NaOH on a 3MM filter paper (Whatman) suspended above the reaction mixture in a plastic well (Kontes). The filter paper was then placed in toluene-Omnifluor (New England Nuclear) and radioactivity determined in a liquid scintillation spectrometer. Specific activity of the enzyme was expressed as pmol CO_2 liberated per min and per mg protein (pmol min^{-1} mg^{-1}). Protein content of the tissue supernatants was determined by the Bradford (1976) dye binding method.

Drugs

The drugs used and their sources were as follows: L-ornithine hydrochloride, (±)-propranolol hydrochloride, and (±)-isoprenaline sulphate obtained from Sigma Chemical Company, St. Louis, MO; terbutaline sulphate and metoprolol tartrate obtained from Ciba-Geigy, Ardsley, NY; Susphrine 1:200 from Cooper Laboratories, Inc., Wayne, NJ; adrenaline (epinephrine) aqueous solution 1:1000 from Eli Lilly and Company, Indianapolis, IN; and prazosin hydrochloride obtained from Pfizer Laboratories, New York, NY. Yohimbine was a gift from Dr William Roeske, University of Arizona.

Results

The adrenoceptor agonists, adrenaline (α_1,α_2, β_1,β_2), isoprenaline (β_1,β_2) and terbutaline (β_2) significantly elevated ODC activity in the foetal mouse heart (Table 1). Susphrine, a sustained release suspension of adrenaline, produced a two fold elevation of ODC activity in the heart. The adrenoceptor antagonists, yohimbine (α_2), prazosin (α_1), metoprolol (β_1) and propranolol (β_1,β_2) failed to reduce control enzyme activity and therefore were not cytotoxic (Table 1). Further, the stimulatory effects of isoprenaline on ODC activity were not inhibited by yohimbine or prazosin, the α_1 and α_2-receptor blockers, respectively. However, the isoprenaline-induced increase in ODC activity was blocked by propranolol, a nonspecific β_1 and β_2-adrenoceptor blocker. Metoprolol (10 mg/kg, s.c.), failed to inhibit the increase in ODC activity induced by isoprenaline or adrenaline. Involvement of the β_2-receptor was demonstrated by the ability of terbutaline, a specific β_2-receptor agonist, to increase ODC activity three fold, an effect that could be blocked by propranolol but not by metoprolol.

Discussion

This study suggests physiological β_2-adrenoceptors in the mouse heart are coupled to the induction of ODC. A β_2 response to catecholamine stimulation is in agreement with the results of Veldhuis, Johannes, Harrison & Hammond (1980) who examined catecholamine modulation of ODC in isolated granulosa cells from immature pig ovarian follicles. Catecholamines produced concentration-dependent increases in ODC activity which were not altered by α-receptor antagonists nor by a β_1-antagonist, metoprolol, but were blocked by propranolol, butoxamine and timolol. In addition, terbutaline, a preferential β_2-agonist, stimulated ODC in a dose-dependent manner. Other authors have reported β_2 coupling to adenylate cyclase activation in human neutrophils (Galant & Allred, 1980) and fat cells (Kather & Simon, 1980), as well as β_2-mediated cyclic AMP increases in mouse epidermis (Duell, 1980) and Ehrlich ascites tumor cells (Onaya, Akasu, Takazawa & Hashizume, 1978). Further, alkaline phosphatase induction in rat liver occurs in response to β_2 catecholamine administration (Mary & Rao, 1981).

In the present studies, adrenaline (α_1,α_2, β_1,β_2),

Table 1 Effects of adrenoceptor agonists and antagonists on ornithine decarboxylase activity in the foetal murine heart

Drug(s)	*Ornithine decarboxylase activity* (pmol min^{-1} mg^{-1} protein)	*% of control*
Control (saline)	12.1 ± 2.5	100
Yohimbine (10 mg/kg)	12.7 ± 1.2	105
Prazosin (1 mg/kg)	13.6 ± 0.9	119
Metoprolol (10 mg/kg)	13.8 ± 1.6	114
Propranolol (10 mg/kg)	12.5 ± 1.4	103
Isoprenaline (10 mg/kg)	49.5 ± 6.3*	409
Adrenaline (1 mg/kg)	37.5 ± 2.8*	301
Susphrine (1 mg/kg)	26.4 ± 3.0*	218
Terbutaline (10 μg/foetus)	37.2 ± 3.2*	308
Isoprenaline + propranolol	6.2 ± 0.9	51
Isoprenaline + metoprolol	43.5 ± 6.0*	360
Isoprenaline + prazosin	39.9 ± 2.6*	330
Isoprenaline + yohimbine	37.6 ± 5.4*	311
Adrenaline + metoprolol	38.1 ± 4.0*	315
Terbutaline + propranolol	9.8 ± 0.8*	82
Terbutaline + metoprolol	49.7 ± 3.3*	411

All experiments were conducted on foetal hearts at 18 days of gestation. Each value represents duplicate determinations of pools of at least 5 foetal hearts. Each value contains data from 5 to 10 separate samples. Drugs administered in combination were at the same doses as for single agents.
Values are expressed as the mean ± s.e.mean.
*Values differ significantly from controls ($P < 0.001$).

isoprenaline (β_1,β_2) and terbutaline (β_2) significantly elevated ODC activity in the foetal mouse heart. Each of these adrenoceptor agonists possesses β_2-receptor stimulating activity. The induction of ODC was not inhibited by the α-receptor antagonists, yohimbine and prazosin. This lack of effect was not surprising since α-agonistic responses generally occur by cyclic AMP-independent mechanisms (Osnes, Christoffersen & Oye, 1973; Exton & Harper 1975; Martinez & McNeill, 1975; Osnes & Oye, 1975; Schümann, Endoh & Brodde, 1975; Verma & McNeill, 1976; Schümann, Motomura, Endoh & Brodde, 1977). Consequently, the receptor mediating ODC activity was β-specific. Both isoprenaline- and terbutaline-induced increases in enzyme activity were blocked by propranolol, a nonspecific antagonist of the β_1 and β_2 receptor subtypes described by Lands, Arnold, McAuliff, Luduena & Brown (1967). Propranolol, in addition to blocking β-receptors, is equipotent to lidocaine as a local anaesthetic on cardiac muscle strips (Morales-Aquilera & Vaughan Williams, 1965). The plasma concentration required for this activity (greater than 10 μg/ml) is 100 times that required for β-receptor blockade (Fitzgerald, 1969). However, Veldhuis *et al.* (1980) have demonstrated that timolol, a non-membrane active β-antagonist (Conolly, Kersting & Dollery, 1976) inhibits adrenaline-stimulated ODC induction in granulosa cells of the pig *in vitro*. Therefore, it appears that propranolol inhibited ODC activity via β-receptor blockade.

Metoprolol was not an effective inhibitor of the adrenaline-, isoprenaline- or terbutaline-stimulated increases in ODC activity in the foetal mouse heart. Likewise, metoprolol failed to inhibit ODC elevation after adrenaline in granulosa cells (Veldhuis *et al.*, 1980). The low membrane stabilizing activity of metoprolol is correlated with its moderate lipophilic properties compared to propranolol (Hellenbrecht, Lemmer, Wiethold & Grobecker, 1973). Metoprolol is a relatively selective β_1-receptor antagonist (Conolly *et al.*, 1976), but in doses exceeding 100 mg per day in human studies, metoprolol also blocks β_2-receptors (Ablad, Borg, Carlsson, Ek, Johnsson, Malmfors & Regardh, 1975; Johnsson, Regardh & Solvell, 1975). The dose used in these studies was approximately 10 times the mg/kg dose which purportedly blocks β_2-receptors in humans, and yet this dose failed to inhibit the agonist-induced ODC activity. Therefore, we conclude that β_1- and not β_2-receptors were blocked by metoprolol in the foetal mouse heart.

Of primary importance in these studies was the finding that only β_2-antagonism inhibited ODC increases in heart. The β_2-receptor was further implicated by the ability of terbutaline, a β_2 agonist, to

induce ODC activity. This induction could be blocked by propranolol and not metoprolol. We suggest, therefore, that the myocardial ODC activity appears coupled to the β_2-adrenoceptor.

This work was supported by USPHS Research Grant HL-20984 from the National Heart, Lung and Blood Institute to D.H.R. and W.R.R. W.R.R. is the recipient of a Research Career Development Award HL-00776 from the National Heart, Lung and Blood Institute.

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(Received July 21, 1981.
Revised October 20, 1981.)