Cardiovascular Research, 1975, **9**, 793–796.

The effect of isoprenaline and propranolol on rat myocardial ornithine decarboxylase[1]

J. WAYNE WARNICA,[2] PAULA ANTONY, KEITH GIBSON, and PETER HARRIS
From the Cardiothoracic Institute, University of London, 2 Beaumont Street, London W1N 2DX

AUTHORS' SYNOPSIS The intraperitoneal injection of isoprenaline in rats caused an increase in myocardial ornithine decarboxylase activity which reached a maximum of about four times the control value one hour after the injection.

The intraperitoneal injection of dl-propranolol had no effect on myocardial ornithine decarboxylase activity. The injection of dl-propranolol 30 min before the injection of isoprenaline almost completely prevented the effect of isoprenaline.

The polyamines, spermidine and spermine, are thought to play an important role in tissue growth (Brewer and Rusch, 1966; Tanner, 1967; Abraham, 1968; Stevens, 1970). Rapid increases in the concentrations of the polyamines have been demonstrated following the initiation of protein synthesis in several tissues (Caldarera *et al*, 1965; Snyder *et al*, 1970; Russell and Lombardini, 1971). This has also been shown to occur during the early stages of cardiac hypertrophy (Caldarera *et al*, 1971; Russell *et al*, 1971; Feldman and Russell, 1972) and after the increased work load of two hours' swimming (Caldarera *et al*, 1974). The *in-vitro* stimulation of cardiac protein synthesis by polyamines has also been demonstrated (Caldarera *et al*, 1974; Gibson and Harris, 1974).

The polyamines are formed from the combination of putrescine and s-adenosylmethionine. Putrescine is derived from the decarboxylation of ornithine (Pegg and Williams-Ashman, 1968). The latter reaction is catalysed by the enzyme l-ornithine decarboxylase (Pegg and Williams-Ashman, 1968). The apparent biological half-life of l-ornithine decarboxylase is very short, 10–15 min in the rat liver (Russell and Snyder, 1969) and 23–26 min in the rat myocardium (Matsushita *et al*, 1972; Warnica *et al*, 1974). The rapid rate of turnover of this enzyme suggests that its concentration may control the rate of polyamine production. The relation of ornithine decarboxylase to protein synthesis may thus be important.

Various stimuli which lead to an increased protein synthesis have been shown to produce a rapid increase in ornithine decarboxylase activity in a number of different tissues (Russell and Snyder, 1968; Jänne and Raina, 1969; Russell *et al*, 1970; Snyder *et al*, 1970; Cavia and Webb, 1972; Mallette and Exton, 1973). In the heart an increased cardiac work produced by aortic constriction, pulmonary arterial constriction or chronic hypoxia has respectively resulted in measurable increases in this enzyme activity at 2–4 h (Russell *et al*, 1971; Matsushita *et al*, 1972), 24 h (Feldman and Russell, 1972) and 4 days (Krelhaus *et al*, 1975). We have recently shown that increasing cardiac work by acute exercise induced an increase in enzyme activity within 30 min (Warnica *et al*, 1974).

The sympathetic-catecholamine system is known to be stimulated under conditions of stress associated with a rapid increase in cardiac

[1] This work was supported by a grant from the Medical Research Council.
[2] Canadian Medical Research Council Fellow.

work. Following aortic constriction in rabbits, Caldarera has demonstrated a transient increase in the myocardial concentration of noradrenaline reaching its maximum after 30 min (Caldarera *et al*, 1971). Isoprenaline stimulates the production of measurable cardiac hypertrophy in rats at doses as low as 1 mg/kg and after as few as four doses (Stanton *et al*, 1969). Propranolol, when given in large doses (20 mg/kg) in conjunction with isoprenaline, will prevent up to 80% of the cardiac hypertrophy which would be expected from the isoprenaline (Stanton *et al*, 1969).

In the present study we have examined the effects of isoprenaline on rat myocardial ornithine decarboxylase. In addition, we report the effects of propranolol on this enzyme and on its response to isoprenaline administration.

Materials and methods

[l-^{14}C] Ornithine monohydrochloride (specific activity 61 mCi/mmol) obtained from the Radiochemical Centre, Amersham, Bucks, UK, was freshly dissolved in water daily to a concentration of 5 μCi/ml. The dl-isoprenaline and dl-propranolol (both obtained from Sigma Chemical Corporation, St Louis, Mo, USA) were dissolved in normal saline so that the final concentration for isoprenaline was 1 mg/ml and for propranolol was 10 mg/ml.

Male albino Sprague-Dawley rats, body weight 150–200 g, were obtained from Fisons Pharmaceuticals Limited, Loughborough, Leics, UK, and kept in our animal house on a MRC 41B diet for at least 7 days before use. For each study only rats of the same age and batch were used. For the first study isoprenaline (2 mg/kg body weight) was given intraperitoneally to the test rats, while control rats received an equal volume of normal saline. Groups of five test and five control rats were killed at selected time intervals by a sharp blow to the head and the hearts removed immediately to ice cold saline (4°C).

In the second study, the rats were divided into three large groups. The first group received propranolol, 20 mg/kg body weight, followed after 30 min by isotonic saline. The second group received propranolol, 20 mg/kg body weight, followed after 30 min by isoprenaline, 2 mg/kg body weight. The third group received saline, followed after 30 min by isoprenaline, 2 mg/kg body weight. All injections were in a volume of 0.4 ml and were given intraperitoneally. The first group acted as a control showing the effect of propranolol and the stress due to injection. The difference in activity between the second and third group demonstrated the effect of propranolol on isoprenaline stimulation of ornithine decarboxylase. Five rats from each group were killed by a sharp blow to the head at intervals of 30 min and the hearts removed immediately to ice cold saline. All subsequent steps were carried out on ice.

After removal, the hearts were homogenized and prepared for assay of enzyme activity as described in a previous paper from this laboratory (Krelhaus *et al*, 1974). Before use in the assay, however, the supernatant was passed through a column of Sephadex G-75 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) to remove the endogenous ornithine (Warnica *et al*, 1974).

The assay of ornithine decarboxylase, based on the method of Russell and Snyder (1969), was carried out as described by Krelhaus *et al* (1974). The protein content of the enzyme source was measured by the method of Lowry *et al* (1951) using bovine serum albumin as standard. Using the protein content, the specific activity of labelled ornithine, and the channels ratio method to determine quenching, enzyme activity was calculated as pmol $^{14}CO_2$ produced/mg protein·h^{-1} incubation at 37°C.

Results

The effect of injecting the rats with isoprenaline (2 mg/kg body weight) is illustrated in Fig. 1. There was a slight rise in enzyme activity after half an hour. After 60 min, the activity was clearly higher in the animals given isoprenaline ($P < 0.005$). The peak rise in activity occurred two hours after the injection and was four times the control values. The enzyme activity then decreased but was still higher than controls 4 h after the injection ($P < 0.005$). The control enzyme activity did not change over this period.

The effects of dl-propranolol and the combined effects of dl-propranolol and isoprenaline are demonstrated in Fig. 2. The administration of isoprenaline alone had an effect similar to that just described, the activity of myocardial l-ornithine decarboxylase increasing to 10 times its control value after 2 hours.

The group of animals killed at zero time for this part of the study served as controls both for the time-course of the effects of isoprenaline and for the animals which had been given dl-propranolol 30 min previously. The activity of

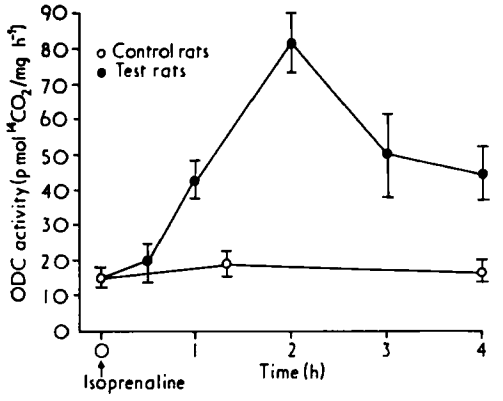

FIG. 1 *Effects of intraperitoneal isoprenaline (2 mg/kg body weight) on ornithine decarboxylase activity (ODC) of rat hearts. Time indicates hours after injection. Values are means ± SE for five hearts.*

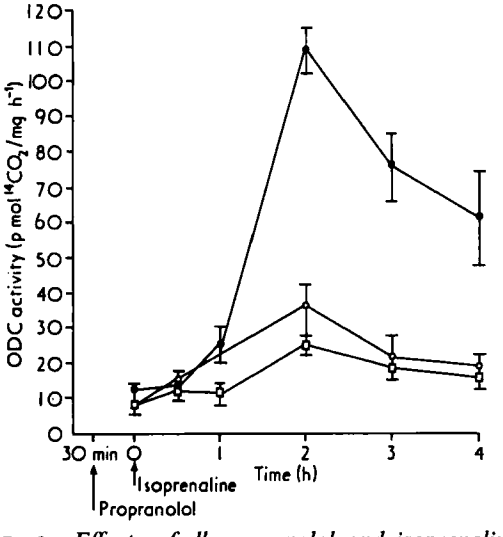

FIG. 2 *Effects of dl-propranolol and isoprenaline on ornithine decarboxylase activity (ODC) of rat hearts. Time is hours after injection of isoprenaline. Note that propranolol was injected 30 min before zero time. Values are means ± SE for five hearts at each point. Group I (□) were injected with propranolol; group II (○) with propranolol plus isoprenaline, and group III (●) with isoprenaline.*

myocardial l-ornithine decarboxylase in these controls was not significantly different from that of the animals treated with dl-propranolol. During the next 4 h there were only small variations in activity in the animals treated with dl-propranolol followed by an injection of saline. As will be seen, however, from Fig. 2, there was a suggested of a small peak of activity 2 h after the injection of saline ($P < 0.05$ when compared with zero time).

The group of animals which received dl-propranolol followed by isoprenaline failed to show the large increase in l-ornithine decarboxylase activity which had been observed with isoprenaline alone. There was a slight peak of activity at 2 h similar to and slightly greater than that found with propranolol followed by an injection of saline. The difference between the two groups treated with dl-propranolol was, however, not significant.

Discussion

The results show an early and considerable increase in the activity of myocardial ornithine decarboxylase in rats injected with isoprenaline. Such a response is similar to that which we have found during and after a 2-h period of swimming exercise in the rat (Warnica *et al*, 1974). An early increase in ornithine decarboxylase activity has also been reported following aortic constriction (Russell *et al*, 1971; Matsushita *et al*, 1972). Both pulmonary arterial constriction and an increased right ventricular load due to hypoxic pulmonary hypertension have been associated with an increased activity of ornithine decarboxylase activity in the right ventricle (Feldman and Russell, 1972; Krelhaus *et al*, 1974). All these conditions are associated with an increased cardiac work. In the doses used, isoprenaline stimulates the force and rate of cardiac contraction, augmenting the cardiac output and oxygen uptake (Innes and Nickerson, 1970) and leading to hypertrophy (Stanton *et al*, 1969). It may, therefore, be that the effects of isoprenaline on ornithine decarboxylase activity are secondary to the increased cardiac work to which it gives rise.

Alternatively the effects of work on ornithine decarboxylase activity may be mediated by the local release of noradrenaline into the myocardium. The experimental conditions used to cause an increased work load on the heart are themselves likely to cause an increase in circulating catecholamines. It seems unlikely, however, that

this would account for the increase in myocardial ornithine decarboxylase activity found under those conditions since the increase is specifically located in the overloaded ventricle.

Catecholamines are known to convert glycogen phosphorylase to an active form via a cascade developing from the adenyl cyclase system. Studies in our laboratory, however, have failed to show any *in-vitro* effect of cyclic AMP and protein kinase on ornithine decarboxylase activity (Warnica *et al*, 1974). Neither isoprenaline nor propranolol had any effect on ornithine decarboxylase activity *in vitro* (Warnica *et al*, 1974).

In the present series of studies propranolol had no effect on myocardial ornithine decarboxylase activity but almost completely prevented the effects of isoprenaline. If the influence of isoprenaline on myocardial ornithine decarboxylase activity is secondary to the increase in cardiac work, it is conceivable that propranolol blocks the effect of isoprenaline on the enzyme simply by inhibiting the increase in mechanical activity of the heart. If, on the other hand, the effect of isoprenaline on myocardial ornithine decarboxylase is a more direct metabolic one, the fact that propranolol blocks it gives a clue to the nature of the receptor system involved.

References

Abraham, A. J. (1968). Studies on DNA-dependent RNA polymerase from Escherichia coli. 1. Mechanism of polyamine-induced stimulation of enzyme activity. *European Journal of Biochemistry*, **5**, 143–146.

Brewer, E. N., and Rusch, H. P. (1966). Control of DNA replication; effect of spermine on DNA polymerase activity in nuclei isolated from Physarum polycephalum. *Biochemical Research Communications*, **25**, 579–584.

Caldarera, C. M., Barbiroli, B., and Morruzzi, G. (1965). Polyamines and nucleic acids during development of the chick embryo. *Biochemical Journal*, **97**, 84–88.

Caldarera, C. M., Casti, A., Rossoni, C., and Visioli, D. (1971). Polyamines and noradrenaline following myocardial hypertrophy. *Journal of Molecular and Cellular Cardiology*, **3**, 121–126.

Caldarera, C. M., Orlandini, G., Casti, A., and Morruzzi, G. (1974). Polyamine and nucleic acid metabolism in myocardial hypertrophy of the overloaded heart. *Journal of Molecular and Cellular Cardiology*, **6**, 95–104.

Cavia, E., and Webb, T. E. (1972). Modified induction of ornithine decarboxylase by factors which affect liver regeneration. *Biochimica et Biophysica Acta*, **262**, 546–554.

Feldman, M. J., and Russell, D. H. (1972). Polyamine biogenesis in the left ventricle of the rat heart after aortic constriction. *American Journal of Physiology*, **222**, 1199–1203.

Gibson, K., and Harris, P. (1974). The effects of polyamines on cardiac protein biosynthesis. *Cardiovascular Research*, **8**, 668–673.

Inness, I. R., and Nickerson, M. R. (1970). Isoproterenol. In *The Pharmacological Basis of Therapeutics*, ed. by L. S. Goodman and A. Gilman, 4th ed., ch. 24, pp. 449–450. Macmillan: London.

Jänne, J., and Raina, A. (1969). On the stimulation of ornithine decarboxylase and RNA polymerase activity in rat liver after treatment with growth hormone. *Biochimica et Biophysica Acta*, **174**, 769–772.

Krelhaus, W., Gibson, K. I., and Harris, P. (1975). The effects of hypertrophy, hypobaric conditions, and diet on myocardial ornithine decarboxylase activity. *Journal of Molecular and Cellular Cardiology*, **7**, 63–69.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.

Mallette, L. E., and Exton, J. H. (1973). Stimulation by insulin and glucagon of ornithine decarboxylase activity in the perfused rat livers. *Endocrinology*, **93**, 640–644.

Matsushita, S., Sogani, R. K., and Raben, M. S. (1972). Ornithine decarboxylase in cardiac hypertrophy in the rat. *Circulation Research*, **31**, 699–709.

Pegg, A. E., and Williams-Ashman, H. G. (1968). Biosynthesis of putrescine in the prostate gland of the rat. *Biochemical Journal*, **108**, 533–539.

Russell, D. H., and Lombardini, J. B. (1971). Polyamines: (1) Enhanced s-adenosyl-l-methionine decarboxylase in rapid growth systems, and (2) the relationships between polyamine concentrations and RNA accumulation. *Biochimica et Biophysica Acta*, **240**, 273–286.

Russell, D. H., Shiverick, K. T., Hamrell, B. B., and Alpert, N. R. (1971). Polyamine synthesis during initial phases of stress-induced cardiac hypertrophy. *American Journal of Physiology*, **221**, 1287–1291.

Russell, D. H., and Snyder, S. H. (1968). Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo and various tumors. *Proceedings of the National Academy of Sciences*, **60**, 1420–1427.

Russell, D. H., and Snyder, S. H. (1969). Amine synthesis in the regenerating rat liver: extremely rapid turnover of ornithine decarboxylase. *Molecular Pharmacology*, **5**, 253–262.

Russell, D. H., Snyder, S. H., and Medina, V. J. (1970). Growth hormone induction of ornithine decarboxylase in rat liver. *Endocrinology*, **86**, 1414–1419.

Snyder, S. H., Kreuz, D. S., Medina, V. J., and Russell, D. H. (1970). Polyamine synthesis and turnover in rapidly growing tissues. *Annals of the New York Academy of Sciences*, **171**, 749–771.

Stanton, H. C., Brenner, G., and Mayfield, E. D. (1969). Studies on isoproterenol-induced cardiomegaly in rats. *American Heart Journal*, **77**, 72–80.

Stevens, L. (1970). The biochemical role of naturally occurring polyamines in nucleic acid synthesis. *Biological Reviews*, **45**, 1–27.

Tanner, M. J. A. (1967). Effects of polyamines on the binding of aminoacyl transfer ribonucleic acid to ribosomes in a yeast system. *Biochemistry*, **6**, 2686–2694.

Warnica, J. W., Antony, P., Gibson, K. I., and Harris, P. (1974). Myocardial ornithine decarboxylase: properties and response to exercise in the rat. (Unpublished observations.)