Involvement of calcium ions in the activation of ornithine decarboxylase by isoprenaline evaluated 'in situ' in the perfused rat heart

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Ornithine decarboxylase activity evaluated during the perfusion of isolated rat hearts by a method 'in situ' was rapidly increased when the hearts were infused with isoprenaline (isoproterenol). Omission of Ca^{2+} from the perfusion medium or the administration of verapamil to the perfused hearts decreased the isoprenaline-stimulated ornithine decarboxylase activity, whereas a marked stimulatory effect was registered when the hearts were perfused with the Ca^{2+} ionophore A23187.

Previous research indicated that ODC activation is mediated in different cell types by Ca^{2+} .

A requirement for Ca^{2+} for the induction of ODC in endothelial (D'Amore & Shepro, 1978) and Chinese-hamster ovary cells has been reported (Costa & Nye, 1978). ODC stimulation also increases in rat hepatoma-cell cultures (Canellakis *et al.*, 1981) and in guinea-pig lymphocytes (Otani *et al.*, 1982) under experimental conditions that elevate cellular Ca^{2+} concentration. Moreover, the research of Gibbs *et al.* (1980) in rat astrocytoma cells and Veldhuis & Hammond (1981) in pig granulosa cells clearly indicates that Ca^{2+} ions are involved in the ODC activation induced by adrenergic compounds. Since cardiac ODC activity is rapidly increased by β -adrenergic agonists (Warnica *et al.*, 1975; Corti *et al.*, 1977; Lau & Slotkin, 1979; Veldhuis *et al.*, 1980), we have studied the possibility that Ca^{2+} ions are involved in the activation of the enzyme when the heart muscle is perfused with isoprenaline (isoproterenol).

To obtain an early indication about the biochemical events involved in the activation of ODC during isoprenaline infusion, we have determined ODC activity by monitoring the production of the $^{14}\text{CO}_2$ evolved from the coronary effluents collected from the perfusate of [$1\text{-}^{14}\text{C}$]ornithine-perfused rat hearts.

Materials and methods

Isoprenaline was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.; ionophore A23187 was

obtained from Calbiochem–Behring, San Diego, CA, U.S.A. Verapamil was from Knoll A.G., Liestal, Switzerland; DL- α -difluoromethylornithine was a gift from Merrell Research, Strasbourg, France. Female Wistar rats (150–200 g), which were fed *ad libitum*, were anaesthetized with diethyl ether. The hearts were excised and rinsed briefly with a cold perfusion buffer before establishment of a retrograde non-recirculating perfusion circuit as described elsewhere (Guarnieri *et al.*, 1978). The perfusion medium, equilibrated with O_2/CO_2 (19:1) at 37°C, contained (mM): NaCl, 115.0; NaHCO_3 , 25.0; KCl, 4.0; KH_2PO_4 , 0.9; MgSO_4 , 1.1; CaCl_2 , 1.5; glucose, 11.0. A perfusate flow of 12 ml/min was maintained throughout the individual experiment. The heart was initially perfused for a 20 min stabilization period. [$1\text{-}^{14}\text{C}$]Ornithine (42.7 Ci/mol; New England Nuclear, Dreieich, W. Germany) was dissolved in the perfusion medium and then infused into the aortic inflow cannula. The radioisotope flow rate was 1 ml/min and the heart received $0.4\text{ }\mu\text{Ci/min}$ for 30 min. Ornithine decarboxylation was determined by monitoring the production of $^{14}\text{CO}_2$ from the coronary effluents. Samples of the perfusates were collected at 1 min time intervals in 25 ml Erlenmeyer flasks containing 0.5 ml of 0.5 M-NaOH/0.125 M-EDTA. The flasks were sealed with rubber serum stoppers equipped with plastic centre wells containing a paper wick impregnated with Protosol (New England Nuclear). The perfusion fluid in the flask was then acidified by injecting 0.4 ml of 40% trichloroacetic acid through the serum cap. The flasks were agitated at room temperature for 30 min, and then released $^{14}\text{CO}_2$, trapped with Protosol, was transferred to scintillation vials containing toluene-based scintillation fluid (containing 0.5% 2,5-di-

Abbreviation used: ODC, ornithine decarboxylase (EC 4.1.1.17).

phenyloxazole). A sample of perfusate collected at zero time before cannulation of the aorta was collected for 1 min and acidified in order to evaluate blank $^{14}\text{CO}_2$ production.

Results

Fig. 1 shows the kinetics of $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ ornithine in the perfused rat heart. In the control experiments, the ornithine decarboxylation remained at 2–3 pmol/min per g. The hearts perfused with isoprenaline exhibited a marked increase in $^{14}\text{CO}_2$ production, which reached a maximum of 10–11 pmol/min per g after 15 min of perfusion. A further increase in $^{14}\text{CO}_2$ evolution was measured in the perfused hearts isolated from rats 4 h after the intraperitoneal injection of isoprenaline. Fig. 1 also shows that the addition of difluoromethylornithine, a known irreversible inhibitor of ODC (Bey, 1978), to isoprenaline-perfused rat hearts strongly decreased the rate of ornithine decarboxylation. At the end of the infusion of $[1-^{14}\text{C}]$ ornithine, the decrease in the rate of production or the washout of the $^{14}\text{CO}_2$ from the heart was rapid. Fig. 2 shows that the activation of ornithine decarboxylation induced by isoprenaline infusion is modulated by the availability of Ca^{2+} ions. In fact, the presence of the calcium ionophore A23187 increased $^{14}\text{CO}_2$ formation in the perfusate, which was particularly evident in the first 10 min of perfusion.

Fig. 1. Effect of isoprenaline on the decarboxylation of $[1-^{14}\text{C}]$ ornithine in the isolated perfused rat heart. Hearts were perfused for 30 min with $[1-^{14}\text{C}]$ ornithine as described in the Materials and methods section ($\blacktriangle$) in the presence of isoprenaline ($\bullet$) or isoprenaline plus difluoromethylornithine (2 mM) ($\blacklozenge$). The hearts removed after 4 h from rats injected with isoprenaline (3 mg/kg) were perfused only with the labelled ornithine ($\blacksquare$). Each point represents the mean of four observations.

Fig. 2. Effect of A23187 and extracellular Ca^{2+} deprivation on $[1-^{14}\text{C}]$ ornithine decarboxylation in the perfused rat heart.

Hearts infused for 30 min with labelled ornithine received isoprenaline alone ($\bullet$) or isoprenaline plus A23187 ($\blacklozenge$) or isoprenaline plus Ca^{2+} -free perfusion medium ($\blacktriangle$). Each point represents the mean of four observations.

Fig. 3. Effect of verapamil on the isoprenaline-induced stimulation of $[1-^{14}\text{C}]$ ornithine decarboxylation in the perfused rat heart.

Hearts were perfused for 50 min with labelled ornithine in the presence of isoprenaline. Verapamil ($10\text{ }\mu\text{M}$) was injected as indicated in the Figure in the experiments shown by ($\bullet$), whereas in the experiments shown by ($\bullet \cdots \bullet$) it was present for another 25 min. Each point represents the mean of four experiments.

Conversely, the omission of Ca^{2+} from the perfusion medium decreased the decarboxylation of

[1- ^{14}C]ornithine stimulated by the isoprenaline. Also, the addition of verapamil to isoprenaline-perfused rat hearts decreased the formation of $^{14}\text{CO}_2$ (Fig. 3). This inhibitory effect was evident during verapamil infusion, but, after its cessation, there was an increase in the metabolic flow of $^{14}\text{CO}_2$ from the perfusate after 10 min.

Discussion

This study indicates that the decarboxylation of [1- ^{14}C]ornithine evaluated in perfused rat hearts by collecting the $^{14}\text{CO}_2$ released from the coronary effluents is stimulated early by isoprenaline. The fact that difluoromethylornithine almost completely inhibits decarboxylation of the labelled ornithine indicates that the large amount of $^{14}\text{CO}_2$ collected from the perfusate derived mainly from the cardiac ODC activity. Thus the modifications of ornithine decarboxylation observed under our experimental conditions can be referred to alterations in ODC activity.

Previous studies indicated that heart ODC can be activated by the β -agonist compounds (Fuller & Hemrick, 1978; Copeland *et al.*, 1982), and in perfusion experiments such stimulatory effect was observed after 5 min of perfusion (Corti *et al.*, 1977). The current work implies that the Ca^{2+} concentration or the consequence of an alteration in the normal intracellular Ca^{2+} distribution is an important facet of the β -agonist-mediated stimulation of ODC. In fact, decreased availability of extracellular sources of Ca^{2+} , or the infusion of verapamil, a potent inhibitor of the uptake of Ca^{2+} by plasma membranes (Langer *et al.*, 1975), strongly decreased ODC activation by isoprenaline. By contrast, the infusion of both isoprenaline and calcium ionophore A23187, which increases cellular Ca^{2+} influx (Reed & Lardy, 1972), enhanced ODC activity.

The mechanism(s) by which Ca^{2+} ions participate in the activation of heart ODC is unknown. Some experiments performed by adding various amounts of Ca^{2+} to heart cell-free preparations before or after exhaustive dialysis did not influence enzyme activity (results not shown). Therefore we can speculate that Ca^{2+} does not seem to be directly involved in the activation of pre-existing enzyme molecules, but more probably the cations participate positively in some process involved in the cardiac activation of ODC by isoprenaline. Moreover, since ODC acti-

vation in heart muscle is an event characteristic in the early stage of cardiac hypertrophy, the present data suggest that a decrease in cell Ca^{2+} availability should have an inhibitory effect on heart growth.

In conclusion, the present experiments indicate that the determination of ODC activity '*in situ*' during heart perfusions is an accurate method which can reveal the rapid activation of the enzyme induced by isoprenaline and its dependence on the availability of extracellular Ca^{2+} .

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