# Phosphoinositide Response and Alterations in Free-Radical Oxidation in Rats with Catecholamine-Induced Cardionecrosis

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Vascular spasm, hypertrophy of cardiac muscle cells, and necrotic changes in the myocardial tissue of rats administered norepinephrine in incremental doses over 14 days were accompanied by a considerable activation of lipid peroxidation and a weakening of antioxidant defense during the first 7 days of exposure to this injurious agent. On day 14, despite the greatly increased load of norepinephrine, the concentrations of lipid peroxidation products and the activity of antioxidant enzymes deviated from their control values to a lesser extent than on day 7. A similar change was shown by the concentration of brain tissue phosphatidylinositol-4,5-diphosphate, a source of second messengers, suggesting that the phosphoinositide system of second messengers is involved in the mechanisms whereby the destructive effects of norepinephrine are mitigated.

**Key Words**: *norepinephrine; myocardium; phosphoinositides; enzymes of antioxidant defense*

An important part in the mechanisms of heart damage may be played by catecholamines, whose excess has been associated with energy deficiency, elevated levels of fatty acids in the myocardium, and the entry of calcium ions into calcium channels, with the result that ischemic hypoxia sets in, accompanied by electrophysiological, morphological, and biochemical changes [2].

Long-lasting hypercatecholaminemia greatly boosts lipid peroxidation (LPO). The intensity of LPO processes is inversely related to the activity of the antioxidant system and, in particular, of superoxide dismutase and catalase. The major effects of catecholamines are mediated by so-called second messengers (SM), including those of the phosphoinositide system, which participates in the organization of cellular responses to a broad range to stimuli including hormones, neurotransmitters, and eicosanoids [5]. Functional changes may therefore be expected to occur in the phosphoinositide system of SM under the action of norepinephrine (NE) and to be associated with metabolic changes in the myocardium. The aim of the present study was to examine this association.

## MATERIALS AND METHODS

Sexually mature female Wistar rats were used. Cardionecrosis was produced by injecting NE intraperitoneally in incremental doses once daily as follows: 1 mg/kg body weight on days 1-3, 2 mg/kg on days 4-7, 3 mg/kg on days 8-11, and 4 mg/kg on days 12-14, for a total dose of 35 mg/kg. Rats were decapitated under general anesthesia 2 h postinjection on days 7 and 14.

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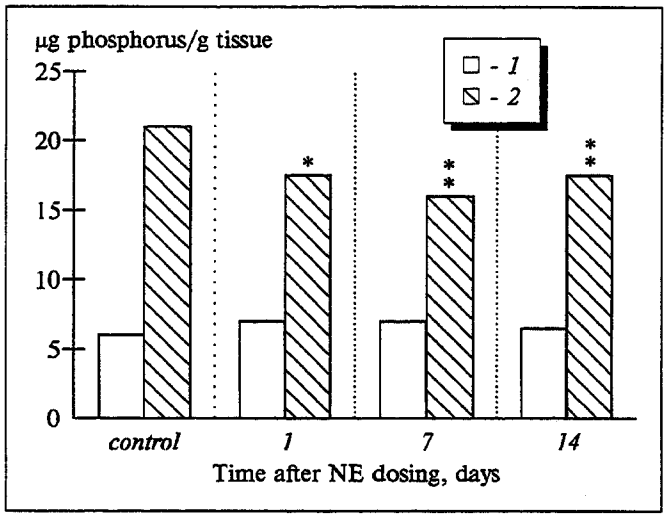


**Fig. 1.** Polyphosphoinositide levels in the brain tissue of rats after NE dosing. *1*) phosphatidylinositol-4-phosphate; *2*) phosphatidylinositol-4,5-diphosphate. *$p<0.05$, **$p<0.02$ in comparison with the control group.

Polyphosphoinositides of brain tissue (phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-diphosphate) were isolated and assayed as detailed by Nakamura *et al.* [6]. LPO products (diene conjugates and Schiff bases) were determined by Plazer's method [7]. Enzymes protecting the myocardium from oxidants (superoxide dismutase and catalase) were assayed as described by Kanvai and Lukoshkin [1]. The procedure described by Merkulov [3] was used for histomorphological examination of the myocardium.

## RESULTS

Examination of the myocardium taken from rats after 7 days of NE dosing revealed congested and spastic vessels, edematous stroma, and foci of hypertrophic myocytes with signs of ischemia, breakdown, and necrosis. After 14 days of dosing, similar, but more strongly marked, structural and necrotic changes were seen; in addition, cardiac myocytes were increased in diameter by 50% ($p<0.01$).

Two hours after the first NE dose, the myocardial concentrations of LPO products were elevated, whereas the activities of antioxidant enzymes were depressed (Table 1): the concentrations of diene conjugates and Schiff bases were 60% and 38% above the control levels, respectively, the activity of superoxide dismutase was 43% lower and that of catalase 28% lower. After 7 days of NE dosing, the concentrations of LPO products were at the same levels as on day 1, while superoxide dismutase and catalase activities had risen but remained below the control levels. By day 14, the concentration of diene conjugates remained high (49% above the control level), whereas that of Schiff bases had dropped to the control value despite the continued NE administration in increasing doses; superoxide dismutase activity was also close to the control value, but catalase activity remained lowered (26% below the control value).

The NE-induced large increases in LPO products, accompanied by diminished activity of antioxidant enzymes, are conducive to the activation of phospholipase hydrolysis and to the detergent action of free fatty acids which damage and may even kill cells, as is indicated by morphological evidence.

The level of phosphatidylinositol-4,5-diphosphate, the main source of SM, was found to be significantly lowered in the NE-dosed rats at all times when it was measured, particularly on day 7, when it decreased to 15.7±2.4 *vs.* 21.3±1.4 μg of phosphoinositide phosphorus/g brain tissue in the control group (Fig. 1). The level of phosphatidylinositol-4-phosphate did not change significantly. A change in the hormonal background elicits additional information flows, and the processing and transduction of this information places new demands on SM. As a result, enhanced hydrolysis of phosphatidylinositol-4,5-diphosphate was induced in the NE-dosed rats, and this led to elevated levels of the SM and hence to the activation of enzymes that are targets for SM [5].

The precise mechanism through which the detected effects of NE are induced in the myocardium via phosphoinositide SM needs further study. However the universal mechanism of the phosphoi-

**TABLE 1.** Effects of Catecholamine-Induced Cardionecrosis on LPO Products and Antioxidant Enzymes in Rat Myocardium

| Time after NE dosing | No. of rats | Diene conjugates, nmol/g lipids | Schiff bases | Superoxide dismutase | Catalase |
|---|---|---|---|---|---|
| | | | arb. units/g tissue | | |
| Control | 10 | 0.26±0.03 | 2.0±1.0 | 662±61 | 27.4±1.3 |
| 2 h | 8 | 0.42±0.02*** | 2.8±0.3* | 376±67*** | 19.8±1.7*** |
| 7 days | 9 | 0.40±0.05* | 2.8±0.3* | 481±45 | 22.9±0.9** |
| 14 days | 9 | 0.39±0.04* | 2.0±0.1 | 567±54 | 20.4±1.3*** |

**Note.** *$p<0.05$, **$p<0.02$, ***$p<0.01$ in comparison with the control group.

nositide response in various tissues [5] suggests that phosphoinositide SM participate in the formation of systems limiting the destructive effects of stress-producing agents. This hypothesis is supported by the similarity of metabolic changes in the myocardium and the phosphoinositide system. It appears that in addition to the functional regulation at the systemic level (brain - myocardium), the adverse effects of an injurious factor such as NE may be blocked in the myocardium itself at the stage of signal transduction to the cell, since the SM system is capable of self-regulation and autooscillations [4].

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