In this connection it is of interest that O_2-uptake by Ehrlich ascites tumor cells is not affected by palytoxin at a concentration of 2 mg/l[16]. An inhibition of membrane ATPase as a mode of action of the toxin seems unlikely[7]. While the crude alcohol extract contains an inhibitor of Na-K-activated ATPase, the bulk of this substance, which has been identified as serotonin[17], is extracted into acetone in a following isolation step, while the precipitate (containing the toxin) has no inhibitory action on Na-K-activated ATPase[7].

Clearly, other methods than microelectrode recording of electrical events would now be required. The question of entrance of the toxin into the cells might be solved, especially since a passive distribution of the positively charged molecule[3,6] would have to result in accumulation within the cells. Measurements of ionic fluxes and ionic concentrations might eventually lead closer to an understanding of the mode of action of this and other toxins found in coelenterates.

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Direct toxic effect of isoproterenol on cultured cardiac muscle cells[1]

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Summary. Isoproterenol at relatively high doses (2.5 mg/ml) has a marked toxic effect on rat heart muscle cells cultivated in vitro. This effect is not prevented by propranolol and therefore is not mediated by beta adrenergic receptors.

Catecholamines administered in large doses can induce myocardial damage. A marked cardiotoxic effect is shown by isoproterenol, a synthetic catecholamine with a selective action on beta receptors[2]. Isoproterenol-induced myocardial necrosis resembles the ischemic necrosis induced by vascular occlusion and has been widely used as a model of 'infarct-like' lesion[3–6]. However, there is no direct evidence for an ischemic pathogenesis of the isoproterenol effect. Reports on coronary vascular changes and thrombosis after injection of isoproterenol[7] have not been confirmed[8]. The current interpretation is that myocardial damage by isoproterenol is due to its strong inotropic and chronotropic action which causes an increased oxygen demand by the heart muscle. This cannot be met by improved blood supply because the drug reduces systemic blood pressure by means of peripheral vasodilatation[8]. An alternative interpretation is that catecholamines exert a direct toxic effect on myocytes. The recent demonstration of myocardial necrosis induced by isoproterenol in the isolated perfused rat heart gives support to this view[9]. We have used cardiac muscle cells in culture for studying the direct cardiotoxic effect of isoproterenol.

Explants of newborn rat heart were grown in gelatin-coated plastic dishes containing Dulbecco medium with 20% horse serum under a continuous flow of 90% air–10% CO_2. Cells dissociated by trypsinization from newborn rat heart were also cultured under the same conditions. 1 week after plating, spontaneous rhythmic contractions could be observed in different areas of the cultures. Isoproterenol at a concentration of 0.01 mg/ml, or higher, increased the frequency of beating. However, at doses of 2.5 mg/ml, or higher, contractions soon became irregular and stopped completely and irreversibly after a few min. In the following h, cardiac cells underwent cell death, as shown by trypan blue exclusion test, and detached from the bottom of the dishes. Small pieces of newborn rat heart incubated for 2 h in culture medium in the presence of 2.5 mg/ml of isoproterenol did not show any sign of growth when transferred to normal medium in culture dishes.

The toxic effect of isoproterenol on myocardial cultures was not prevented by propranolol, a beta blocking agent. In fact, pieces of newborn rat heart preincubated with propranolol at 0.5, 1.5 or 2.5 mg/ml for 15 min before addition of isoproterenol showed no growth in culture. By contrast, propanolol alone did not interfere significantly with cardiac cell proliferation. These findings indicate that the cardiotoxicity of isoproterenol in this in vitro system is not mediated by beta adrenergic receptors. It is possible that oxidation products similar to adrenochrome are responsible for the direct toxic effect of isoproterenol, as suggested by Yates and Dhalla[9]. We have observed a rapid shift in the absorption spectrum of isoproterenol after incubation at 37 °C in the oxygenated culture medium, with a decrease of the 205 nm peak and an increase of the 225 nm peak within 10 min of incubation. These results on in vitro systems support the view that a direct cardiotoxic effect may contribute to the pathogenesis of myocardial necrosis induced by isoproterenol in vivo. Indeed, it has been reported that the isoproterenol effect in vivo is only partially prevented by propranolol[10]. The demonstration of extremely rapid permeability alterations of the sarcolemmal membrane in cardiac muscle cells after infusion of norepinephrine or isoproterenol[11] is also consistent with this interpretation.

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