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# Effects of propranolol on the biochemical modifications induced by a β-adrenergic drug in ischemic hearts

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It has already been demonstrated that the control by propranolol of the cardiac activity stimulated by a β-adrenergic drug [1, 2] was accompanied by the return to basal values of the cellular oxidations supplying the myocardium with the major part of the additional energy produced: the increase in oxygen consumption determined from coronary flow and arteriovenous difference in oxygen content is suppressed by propranolol [2, 3]. The excess substrates oxidized in these circumstances are circulating nutritive substances, glucose, lactate and free fatty acids (FFA), but chiefly FFA in hearts *in situ* [4, 5, 6] or similar substances originating in the cardiac tissue itself from the hydrolysis of glycogen [3–7] and triglycerides [8–10].

But the anaerobic breakdown of glycogen also represents a source of additional energy which is certainly the most rapidly available under any conditions and is the most important when the oxygen supply is restricted by obstructive lesions of the coronary vessels. The predominance of anaerobic processes in supplying energy, when catecholamine release and ischemia combine their effects, may cause sufficient alterations in metabolite concentrations to promote disorders such as thoracic pain. Consequently, it was of interest to investigate whether propranolol attenuated these alterations as well as the stimulation of the oxidative metabolism.

The study was carried out on hearts *in situ*, in dogs weighing 20–28 kg, that had not been given any food for 12 hr. The dogs were anesthetized with barbiturates (25 mg/kg of sodium thiopental intravenously injected to which 5 mg/kg of sodium pentobarbital were added 10 min before the beginning of the test period). All were submitted to ischemia for 45 min. In addition to the ischemia, from the 15th min, six received a perfusion of isoproterenol ($1\ \mu g \cdot kg^{-1} \cdot min^{-1}$) and six the same perfusion associated with the administration of propranolol (0.2 mg/kg intravenously injected and 0.2 mg/kg perfused over 30 min).

The ischemia desired was an incomplete one, analogous to that responsible for angina pectoris, i.e. eliciting a relative deprivation of oxygen and nutritive substances as compared with requirements. Considering the difficulty of initiating an ischemia stable at a given level, which had, furthermore, to be strictly identical in both groups of animals, the blood flow was alternately totally interrupted and left totally free for 2 min by means of a thread placed loosely round the left coronary artery just before its division into two branches, interventricular and circumflex arteries.

Sampling of the myocardium, required for the determination of the main substrate content, planned to be carried out several times in the course of an experiment, 10 min before and 15, 20, 30 and 45 min after the beginning of the test, was made possible by an extracorporal circulation system [11]. After opening and removing a large part of the right thoracic wall, the venae cavae blood flow was diverted to an extracorporal circuit including oxygenator, peristaltic pump and heat exchanger and returned to the animal via the abdominal aorta centrally cannulated. In this way, it was possible to take samples of the left ventricle external wall from its total thickness using the 'drill biopsy' technique [12], the quantitative determinations of lactate [13], glycogen [14], triglycerides [15] and FFA [16] being subsequently performed on the subendocardial and subepicardial layers after separation [11]. With regard to FFA, the analysis was made according to Duncombe's method [16], which is unspecific, because the aim was only to investigate the modification of the overall FFA concentration. But it was made on 200 μl of tissue extract after neutralization instead of being made on 500 μl of plasma. Between sampling and homogenizing, the myocardial fragments were kept in liquid nitrogen in which they had been immersed as soon as obtained (in less than 10 sec, in order to avoid glycogenolysis and lipolysis especially).

The statistical study was made between control and test values using Student's *t*-test.

*Effects of isoproterenol on ischemic hearts.* The heart rate acceleration due to isoproterenol was notably less considerable under the ischemic than normal conditions: the maximal increase was from 142 ± 12 (S.E.M.) to 163 ± 8 beats per min (N.S.) in the former case and from 123 ±

6 to 191 ± 6 ($P < 0.001$) in the latter.

The evolution of the metabolite concentrations in the subendocardial and subepicardial layers appeared to be similar, the difference never being statistically significant.

In spite of the moderate stimulation of the cardiac activity induced by isoproterenol on ischemic hearts, the lactate level rose significantly over that when the ischemia intervened alone (Fig. 1). However, the fall in glycogen content was not more marked under the combined influence of ischemia and isoproterenol (Table 1). Although ischemia does not act like isoproterenol on lipid metabolism, but rather in the opposite direction [10], the FFA concentration declined gradually in the subendocardial layer of ischemic hearts submitted to isoproterenol. No variation was observed in the triglyceride content of the subendocardial layer, the only one in which it was determined, because of the adipose tissue involved in the muscle of the subepicardial layer.

*Effects of propranolol simultaneously administered.* In the presence of propranolol, isoproterenol did not induce any rise in the heart rate which even fell below its control values from 123 ± 9 to 91 ± 9 beats per min ($P < 0.01$) (Fig. 1). The prevention of the heart stimulation had obvious consequences on the lactate production in either layer, but chiefly in the subepicardial layer where the concentration never exceeded that attributable to the ischemia and even remained below it (Fig. 1).

As isoproterenol then failed to increase the frequency and presumably the force of heart contractions because its chronotropic and inotropic actions are generally parallel, no effect was to be expected on glycogenolysis and the fall of glycogen content was not effectively larger than under the influence of the ischemia alone (Table 1). But, whether propranolol antagonized the cardial functional effects of isoproterenol or not, the reduction of the FFA content which occurred in the subendocardial layer was of the same amplitude.

The blockage by propranolol of isoproterenol cardiac functional effects, which appeared to be as complete in ischemic as normal hearts and even to induce a slight fall below the control values, is of course associated in the former as in the latter [2–4] circumstances with a return of the oxygen consumption to these values.

Therefore, the reduction in lactate content of heart muscle, in agreement with that reported by Deltour [3] and Ichihara and Abiko [7] is not to be attributed to the acceleration of its degradation, but to the slowing down of its appearance and suggests an actual inhibition of the anaerobic glycolysis. However, in our experiments, glycogenolysis did not prove sensitive to isoproterenol or propranolol, presumably because the ischemia started prior to drug administration was sufficient to lower the glycogen content to a low level, little consistent with a further lowering. It is known, in fact, that even moderate ischemia is responsible for a glycogenolysis approaching maximum [17, 18]. But, in borderline ischemias, isoproterenol would not lose all ability to induce the glycogen breakdown and an antagonistic effect of propranolol could then be evidenced [3, 7].

The results concerning lipid metabolism are more unexpected. No reduction in the triglyceride content arose from isoproterenol administration, whereas the mobilization by β-adrenergic drugs of heart muscle triglycerides seems well established [8–10]. But the investigations have been, as a rule, performed on hearts *in vitro* and the effects of isoproterenol in this respect are by no means comparable *in vitro* and *in vivo* [19]. Nevertheless, it is surprising that the isoproterenol-induced decrease in FFA concentration persists under the ischemic conditions as ischemia tends to raise this concentration by oxidation deficiency [10]. In fact, the restriction in the supply of exogenous FFA is compensated for by recourse to endogenous FFA. But it is also and still more surprising that propranolol does not prevent the decrease in FFA concentration while it diminishes the heart energy requirements. In fact, it aggravates

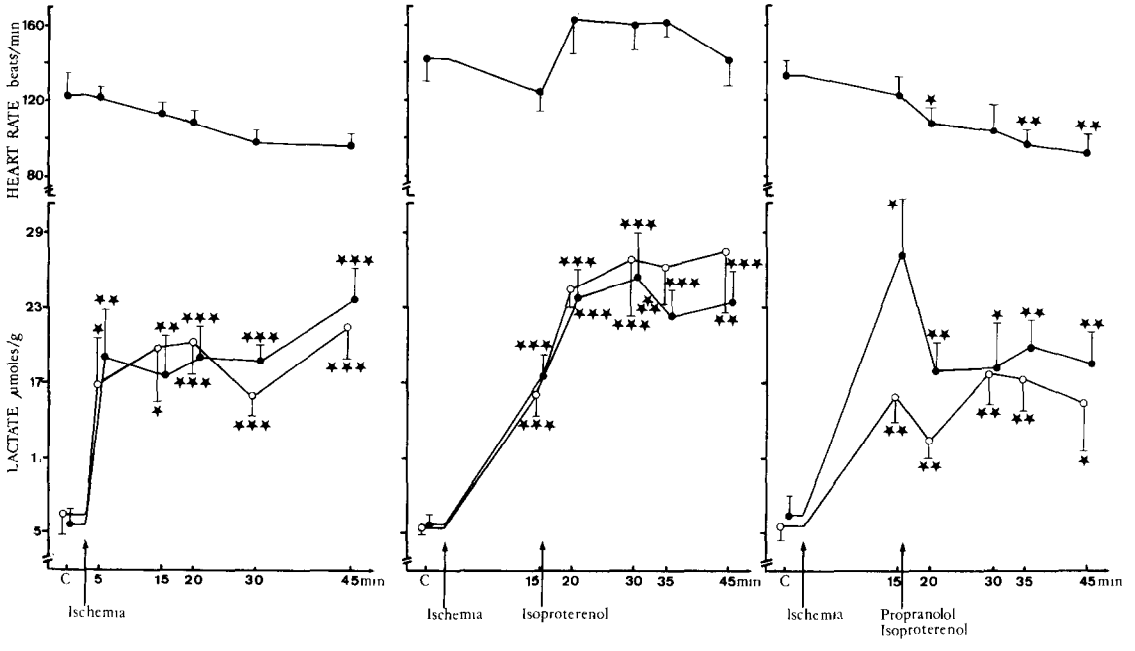


Fig. 1. Variations of heart rate and lactate concentration (μmoles/g wet wt) in the subepicardial (○) and subendocardial (●) layer in dogs submitted to ischemia, isoproterenol under ischemic conditions (administered 15 min after the beginning of ischemia), propranolol and isoproterenol under the same conditions. Mean values ± S.E.M. One, two and three asterisks refer to the significance at the 5, 1 and 0.1% levels, respectively.

Table 1. Variations of glycogen and free fatty acid (FFA) concentrations (mg/g and μmoles/g wet wt, respectively) in the subepicardial and subendocardial layers and of triglyceride (TG) concentration (μmoles/g wet wt) in subendocardial layer in dogs submitted to ischemia, isoproterenol under ischemic conditions (administered 15 min after the beginning of the ischemia), propranolol and isoproterenol under the same conditions*

| | Ischemia | | | | | Ischemia and isoproterenol | | | | | | Ischemia and propranolol–isoproterenol | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | 15 min | 20 min | 30 min | 45 min | C | 15 min | 20 min | 30 min | 35 min | 45 min | C | 15 min | 20 min | 30 min | 35 min | 45 min |
| GLYCOGEN | | | | | | | | | | | | | | | | | |
| Subepi. | 5.52 ± 0.65 | 3.55 ± 0.29† | 3.85 ± 0.24† | 4.41 ± 0.46 | 3.13 ± 0.61† | 5.56 ± 0.50 | 5.46 ± 0.74 | 4.69 ± 0.48 | 3.54 ± 0.63† | 4.24 ± 0.93 | 3.77 ± 1.13 | 5.76 ± 0.42 | 4.69 ± 0.42 | 4.50 ± 0.48 | 4.55 ± 0.55 | 4.51 ± 0.54 | 5.08 ± 0.38 |
| Subendo. | 7.56 ± 0.55 | 4.81 ± 0.40† | 4.97 ± 0.31† | 4.37 ± 0.41§ | 3.76 ± 0.52§ | 7.46 ± 0.62 | 6.12 ± 0.95 | 4.70 ± 0.68† | 5.48 ± 0.85 | 4.89 ± 0.82† | 3.50 ± 0.79‡ | 7.40 ± 0.70 | 5.60 ± 0.48 | 5.42 ± 0.50† | 5.21 ± 0.71† | 5.05 ± 0.65† | 4.39 ± 0.43‡ |
| FFA | | | | | | | | | | | | | | | | | |
| Subepi. | 12.85 ± 1.17 | 11.94 ± 1.26 | 12.36 ± 0.99 | 13 ± 1.19 | 12.53 ± 1.66 | 18.28 ± 1.17 | 17.06 ± 1.86 | 17.32 ± 1.88 | 15.78 ± 1.89 | 15.72 ± 1.74 | 16.26 ± 2.43 | 19.83 ± 0.88 | 16.17 ± 0.54† | 17.01 ± 0.37† | 16.09 ± 0.71‡ | 16.32 ± 0.94† | 16.35 ± 0.80† |
| Subendo. | 11.42 ± 1.06 | 11.23 ± 1.20 | 11.23 ± 1.14 | 10.99 ± 1.20 | 10.86 ± 1.25 | 18.12 ± 0.60 | 16.52 ± 1.58 | 14.83 ± 1.36† | 15.19 ± 0.88† | 15.05 ± 1.19† | 12.70 ± 2.48† | 18.92 ± 0.76 | 17.53 ± 2.08 | 15.50 ± 0.81† | 15.41 ± 0.93† | 16.71 ± 1.18 | 14.71 ± 0.44§ |
| TG | | | | | | | | | | | | | | | | | |
| Subendo. | | | | | | 5.63 ± 0.42 | 5.30 ± 0.36 | 5.28 ± 0.70 | 6.92 ± 1.49 | 4.50 ± 1.15 | 5.38 ± 0.10 | 5.75 ± 0.83 | 6.35 ± 1.20 | 4.95 ± 1.09 | 5.32 ± 1.08 | 4.13 ± 0.87 | 5.87 ± 1.35 |

* Results expressed as means ± S.E.M. C = control.
† Significant at 5% level.
‡ Significant at 1% level.
§ Significant at 0.1% level.

the restriction in the supply of exogenous FFA by opposing the increase in the arteriovenous concentration difference elicited by isoproterenol [4–6].

In conclusion, propranolol impairs, to some extent, the rise in lactic concentration within myocardial tissue due to the combined action of moderate ischemia and a $\beta$-adrenergic drug, but it exerts a lesser influence on glycogenolysis and no influence on the reduction of free fatty acid content. The mechanism of the prevention by propranolol of angina pectoris attacks probably lies in limiting the increase in lactic acid production and decrease in pH within myocardial tissue, due to the combined action of moderate ischemia and a release of catecholamines [20–22].

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# Relationship between glycolysis and proliferation of L 1210 cells *in vitro*: effect of a new pharmacological effector: RA-233

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In most *in vitro* cultures, cells are highly dependent on glucose consumption for generation of metabolic energy, although under certain conditions, glutamine can also provide such a source [1]. The part glucose plays is especially clear in the case of cancer cells, which are characterized by intense glycolytic activity [2] that can be considered a factor in tumoral growth [3].

It is customary to express glucose consumption as a function of the number of cells counted at the end of incubation [4]. However, this cannot take account of cell growth kinetics during long-time culture which are often necessary for pharmacological purposes. We have thus attempted to describe glucose consumption by a mode of expression which takes greater account of cell proliferation, and is not dependent on time of incubation or number of cells seeded. This model has been applied to study the metabolic effects of a new anticancer drug on L 1210 cells.

## *Methods*

*Cells and media.* Cell culture conditions have already been described [5] but will be recalled here briefly: L 1210 cells were incubated in Dulbecco's modified Eagle medium containing 10% foetal calf serum supplemented with antibiotics, bicarbonate and glutamine (GIBCO reagents). Final volume in microtitration plates was 0.2 ml, in a water-saturated atmosphere containing 5% $CO_2$. Initial cell concentrations were $2 \times 10^5$ cells/ml and 5000 cells/ml for 24- and 48-hr cultures, respectively. A few 48-hr cultures were also grown in 25 $cm^2$ flasks, the initial concentration being 25,000 cells/ml. The absence of mycoplasm contamination was verified by Flow Laboratory Kit (Ref. 5.070).

The pharmacological effector used was RA 233 (Boehringer-Ingelheim Laboratories) or 2,6-bis-(diethanol amino)-4 piperidino-pyrimido (5,4-d)-pyrimidine, some of whose effects on cancer cells have already been described [6]. Experimental solutions were prepared from a $2 \times 10^{-2}$ mol/l solution in N/50 hydrochloric acid, diluted as required in the culture medium. Control cultures without effector were grown concomitantly. All experiments were carried out in triplicate.

*Cell counts.* Cells were counted in a Malassez hematimeter and the lethality was evaluated by the loss of cell refringence observed with a phase contrast microscope.

The number of cell doublings ($n$) between times $t_1$ and $t_2$ was calculated according to the following formula:

$$n = \frac{\log N_2 - \log N_1}{\log 2},$$

where $N_1$ and $N_2$ were the number of cells measured at times $t_1$ and $t_2$, respectively.