Calorigenic Effects of Isoproterenol During Production of Myocardial Necrosis in the Rat[1] (37546)

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Isoproterenol (1-[3,4-dihydroxyphenyl]-2-isopropylaminoethanol), a powerful β-adrenergic stimulator, has been shown to produce, when administered in various doses to the rat or other experimental animal, cardiac lesions which closely resemble human myocardial infarction (1). The necrotizing properties of this agent, and its mechanism of action, are currently being investigated under a wide variety of experimental conditions.

The severity of isoproterenol-induced cardiac necrosis bears a relationship with dosage (1), sex, body weight and strain of the animal (2), thyroid status (3), diet and body composition (4), and also with the ambient temperature and heat insulation (5, 6). These observations, although seemingly unrelated, suggest a possible relationship between the metabolic status of the subject at the moment of the cardiac insult, perhaps influenced by acutely altered energy exchanges, and the necrotizing effects of the drug.

Data from the literature on the calorigenic effects of isoproterenol are scarce (6). The drug can increase the animal's oxygen consumption and in this respect its potency is greater than that of epinephrine or norepinephrine (7). Early measurements of the effects of isoproterenol upon the metabolism of rats were done by Waterman (8), who, however, had to resort to indirect estimates of the simultaneous influence of spontaneous muscular activity during the determination. Oxygen consumption and respiratory quotient of rats have been seen to rise in the early hours following a large dose of isoproterenol; the Aa. state that the motility of the animals was not affected (9). Others have resorted to anesthesia (10–13), spinal severance (14) or restraint (7) in order to eliminate spontaneous locomotion during the test.

The availability of an automatic metabolic apparatus which permits the continuous and simultaneous registration of oxygen consumption, spontaneous muscular activity and several respiratory parameters of the intact, freely moving animal confined in a thermocontrolled chamber (15), has enabled us to measure and precisely quantitate the acute and delayed calorigenic effects of the drug as related in time with the development of the cardiac necrosis.

Materials and Methods. Metabolic apparatus and method have been previously extensively described (15, 16). Briefly, the operation of this automatic apparatus, which is of the indirect, semi-open variety, is based on the principle of the oil-demand valve: small O_2 bubbles of known size, traverse a layer of paraffin oil and become available to the animal whenever enough negative pressure (2–3 mm H_2O) is created in the side of the valve which communicates with the metabolic chamber. A continuous, electronically transduced and amplified record of the extremely small pressure and plethysmographic changes is obtained which allows minute by minute quantification of the rate of O_2 consumption of the animal, its respiratory characteristics (rate, rhythm, depth) and the spontaneous muscular activity (SM A) exhibited during the run. From the record of an experiment lasting several hours, values of "least observed metabolic rate"

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(LOMR) and "minimal observed metabolic rate" (MOMR) are subsequently derived, which have been shown (17) to be metabolic parameters more consistent and meaningful than the conventional "basal metabolic rate." A value of "minimal calculated metabolic rate" (MCMR) is also obtained utilizing appropriate equations previously derived from the data of a large number of control determinations and relating rate of O_2 consumption with different levels of SMA (17). MCMR represents the theoretical O_2 consumption of the animal at any moment of the run, unbiased by the metabolic effects of its movements, however slight and fugacious (18). With normal, placid animals in "basal" conditions, MOMR and MCMR should coincide; on the other hand, deviations of MCMR over MOMR represent the "net" metabolic effects of endogenous or exogenous calorigenic stimuli, such as catecholamines, salicylates (19), food (20) or other agents.

Two groups (A and B) of male adult albino rats (Simonsen and Holtzman strains, respectively) were utilized; all metabolic determinations were performed in the morning on overnight-fasted animals. Freshly prepared D,L-isoproterenol hydrochloride (Sigma Chemical Co.), dissolved in bacteriostatic water (20 mg/ml), was administered as a single, sc injection (40 mg/kg body wt). The controls received equal volumes of solvent. A typical determination lasted about 300 min, the first 20–30 of which were utilized for thermal equilibration, and the next 70–90 constituted the control period prior to injection. A total of 3680 min of actual record were individually analyzed, quantified for oxygen consumption, temperature changes and SMA, and statistically evaluated for this study. The animals were sacrificed after 24 or 48 hr and the extent of myocardial damage was estimated according to the scale of severity suggested by Rona *et al.* (1).

Results. Within 10–12 min after the isoproterenol administration, the rats exhibited the characteristic symptoms of distress: panting, lying fully extended on one side or back, often with pink, viscous fluid visible at the nostrils and eyes. Rectal temperature, measured in a series of similarly treated rats (unpublished data), increased 2–3° and returned toward normal, following a time course similar to that of the changes of O_2 consumption.

Figure 1 shows the results of a typical experiment. In spite of the intense muscular

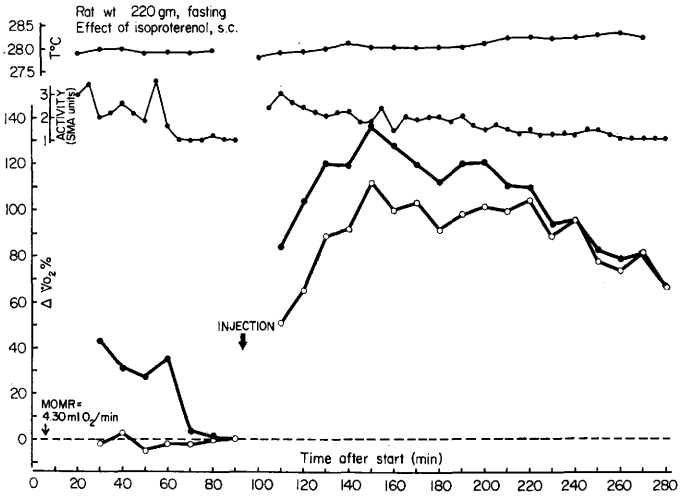

FIG. 1. Acute effects of a single injection of isoproterenol on the total (●) and the net (○) percentage changes of oxygen consumption above minimal observed metabolic rate (MOMR). Spontaneous muscular activity is shown in SMA units (see text). Upper tracing: temperature inside metabolic chamber. Note the almost perfect identity between MOMR and MCMR during the control period, although high SMA was present most of the time.

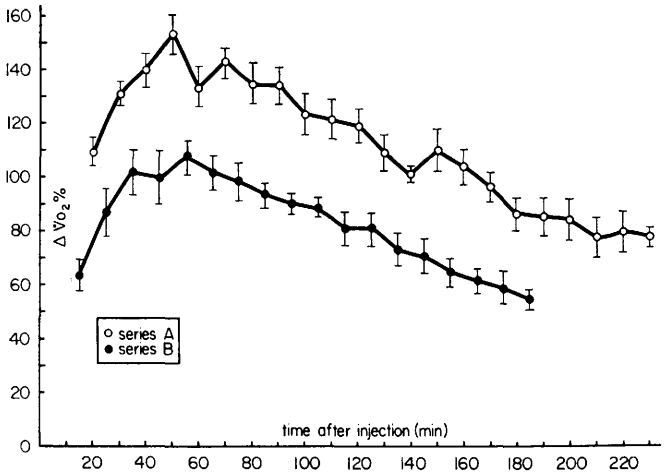

FIG. 2. Mean net percentage changes of oxygen consumption (± SEM) over MOMR after injection of isoproterenol in two series of experiments. Series A: $N = 8$, age 73 days, mean body weight 324 ± 9 g. Series B: $N = 5$, age 58 days, mean body weight 212 ± 2 g.

activity registered during the control period, MCMR values (open circles) essentially coincide with the MOMR of this rat (4.30 ml O_2/min). Following the injection of the drug, a rapid increase in both total and net O_2 consumption occurred. The animal was restless during the early part of the postinjection period, as evidenced by the high levels of recorded activity, causing the discrepancy between observed and calculated metabolic rates, which gradually lessens as the animal ceases moving. The curve drawn through the open circles thus shows changes in O_2 consumption due entirely to the experimental procedure and unaffected by the metabolic effect of SMA.

Average curves of percentage changes in net O_2 consumption in both series, as a function of time, are shown in Fig. 2. As evidenced by the small standard errors, individual experiments produced changes which were remarkably reproducible, although each rat exhibited vastly different levels of spontaneous activity during the run and consequently of total metabolic rate. The resulting curves of net O_2 consumption for both series of determinations are relatively smooth and essentially parallel. The metabolic rate quickly rose to a peak (approximately + 150% in the heavier and + 110% in the lighter series, respectively) between 30–60 min after treatment and then began to decline. At termination of the experiment, 180–230 min after injection, control levels of O_2 consumption were not yet reached. Animals tested 24 and 48 hr after the isoproterenol injection exhibited a resting metabolism (MO MR) which was not different from their controls (Fig. 3), with the possible exception of one 24-hr value. However, when these same

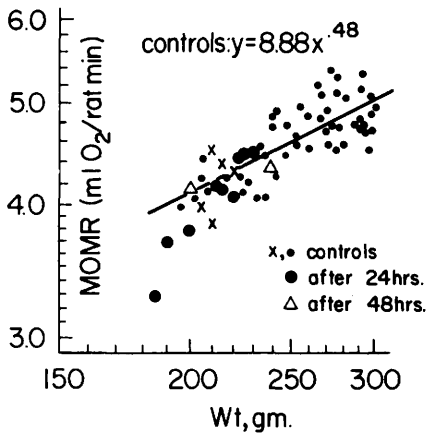

FIG. 3. Double logarithmic plot and regression line of MOMR as a function of body weight in control rats ($N = 50$) of series B. The graph also shows MOMR values obtained during the control period preceding isoproterenol injection (×) and 24 and 48 hr postinjection values.

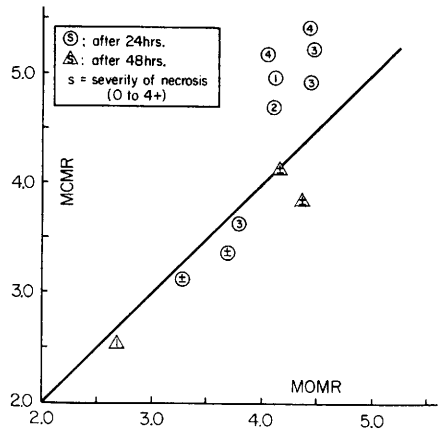

FIG. 4. Comparison between minimal calculated and minimal observed metabolic rates (MCMR vs MOMR) of 12 cardiopathic rats, 1 and 2 days after isoproterenol injection. The degree of myocardial necrosis, subsequently verified at autopsy, is indicated within the symbols. Note the deviations from the identity line ($y = x$) in the heavier and most severely affected animals.

rats engaged in spontaneous bouts of activity during the test, their averaged net metabolic rate (*i.e.*, mean MCMR) did not always coincide with MOMR (Fig. 4): heavier and more severely cardiopathic rats generally yielded MCMR values clearly higher (6 cases out of 9 at 24 hr) than their respective MOMR levels.

Discussion. The accuracy and reproducibility of results obtained with the method employed in this study are evidenced by the striking similarity, smoothness and small variations of the curves describing the acute calorigenic effects of isoproterenol upon two groups of rats differing in strain, body weight and age. The equivocal responses obtained with less sensitive techniques may be attributed in part to the inability to detect and quantitate the spontaneous muscular activity of the subject and to account for it in the interpretation of the data. Muscular movements, however slight and hardly detectable at visual observation, exert a significant influence upon the total metabolic rate of the animal (17) and can be so overwhelmingly preponderant when associated with weak calorigenic effects of some pharmacologic agents to practically obscure them (18, 19). On the other hand, anesthesia, forcible restraint, motor denervation and other stressful interventions, aside from their known effects on the extent and progress of the cardiac damage (21, 22), create such abnormality that the gaseous exchanges of the animal must be undoubtedly affected. Contrary to the experience of others (8, 14), immediately following injection of isoproterenol, the intact, awake, freely moving rat exhibits a period of marked restlessness which coincides with the greatest metabolic action of the drug, which, in our experience, at the large dose employed, reaches its peak in less than 1 hr and steadily decreases in the subsequent hours.

The metabolic response was greater in the older and heavier rats. Increased weight and age are known to make animals more sensitive to the cardiotoxic effects of isoproterenol (2, 4). It may be reasoned that the higher metabolic response to the drug in the heavier, older group, by producing a greater demand for oxygen, enhances the relative myocardial ischemia, which is purported to be responsible for the isoproterenol cardiopathy (1, 23). Since, however, the drug dose was calculated on a straight body weight basis (*i.e.*, mg/kg body wt), and, in the rat, the organs most affected by isoproterenol (heart, liver) obey the allometric law, contributing less to total body weight in the heavy than in the light animals (24), the heart and liver of rats in the heavier group received a relatively greater amount of the drug. This could be the reason for both the larger oxygen consumption and the more severe cardiotoxic effects of isoproterenol observed in the heavier animals.

The toxicity of isoproterenol has been shown to vary directly with the ambient temperature to which the rat is exposed immediately following injection, and it has been suggested that the thermogenic effects of the drug may be in part responsible for its lesion-producing properties (6). The period of maximum calorigenic response essentially coincided with early cardiac ionic changes which may be involved in the production of the lesions (25). It is noteworthy that agents which decrease oxygen consumption (26, 27)

and which reduce the cardiac ion shifts following isoproterenol administration (28) are beneficial in the treatment of angina pectoris and also protect animals from the cardiotoxicity of isoproterenol. The fact that treatments exerting protective effects against isoproterenol influence both oxygen consumption and cardiac ionic equilibrium suggests that these two variables may be linked.

The metabolic rate of the rats treated with isoproterenol had returned to control values by 24 hr (Fig. 3). Spontaneous muscular activity of rats with severe cardiac lesions elicited increases of oxygen consumption which were greater than those of control rats at the same level of activity. In control rats and in those with hearts not severely damaged by isoproterenol, the values of MCMR and MO MR were essentially identical. However, when the metabolic increase normally associated with that level of activity in which the animal has engaged is subtracted from its total oxygen consumption, the resulting MC MR of the rat with cardiac necrosis is greater than its MOMR (Fig. 4). The caloric efficiency for relatively minor degrees of muscular activity (*i.e.*, changing position within the metabolic chamber, grooming, etc.) thus seems to be less than normal in rats with isoproterenol-induced cardiac necrosis. This method of evaluation of the presence, and possibly of the extent of myocardial damage in the living animal could conceivably be employed in other experimental conditions where it would be convenient to know, prior to autopsy, whether an animal has myocardial disease. Thus, a diagnostic and prognostic value could be hypothesized for this metabolic approach to the experimental cardiopathy.

Summary. Rate of oxygen consumption and spontaneous muscular activity (SMA) were measured in two series of rats of different body weights, treated with a single sc dose (40 mg/kg body wt) of isoproterenol. Minimal calculated metabolic rate (MCMR: "net" O_2 consumption unbiased by SMA) reached, within 1 hr, peaks up to +150% in heavier and 110% in lighter rats, above minimal observed metabolic rate (MOMR: resting metabolism of the animal). After 24 and 48 hr, when isoproterenol-induced cardiac lesions were evident, MOMR had returned to normal. However, episodes of SMA in rats with severe cardiopathy elicited disproportionate increases of oxygen consumption (with MCMR significantly higher than MOMR), suggesting caloric inefficiency of the treated animals.

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