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THE EFFECT OF ANTI-LIPOLYTIC AGENTS ON ISOPRENALINE-INDUCED MYOCARDIAL NECROSIS IN THE RAT

N. WOOLF, E. STEEN, D. ROSEN, R. TRICKEY, P. LONG, A. L. MILLER AND P. M. ROWLES

From the Bland-Sutton and Courtauld Institutes, The Middlesex Hospital Medical School, London W1 and the Biophysics Laboratory, Chelsea College, London

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Summary.—The effect of inhibiting isoprenaline-induced lipolysis on the degree of damage produced in the rat myocardium by this amine has been investigated by pre-dosing rats with the anti-lipolytic agent 5-fluoro-nicotinic acid. The degree of myocardial necrosis produced in animals given isoprenaline alone and those pre-dosed with the anti-lipolytic agent was measured by the use of an automated flying spot microscope to show absence of formazan from dead muscle fibres in sections treated to demonstrate succinic dehydrogenase. The use of the anti-lipolitic considerably reduced the degree of myocardial damage produced by a standard dose of isoprenaline bitartrate. This was associated with an inhibition of the post-isoprenaline rise in plasma free fatty acid levels. The results are discussed in relation to the possible protective roles of the lowering of plasma free fatty acid levels and inhibition of the adenyl cyclase system at the plasma membrane of the myocardial cell produced directly by the anti-lipolytic.

CONVINCING evidence exists that myocardial ischaemia is associated with a release of endogenous catecholamine in the myocardium and a concomitant increase in plasma and urine catecholamine levels (Ceremuzynski, Staszewska-Barczak and Herbaczynska-Cedro, 1969; Gazes, Richardson and Woods, 1959). Such local and systemic increases in catecholamine levels could affect the performance and survival of myocardial fibres in a variety of ways (Fitzgerald, 1972). In this communication attention is focused on only one of these: the breakdown of triglyceride in adipose tissue and the possible deleterious effects on the myocardium which may arise from the resulting elevation of plasma free fatty acid levels.

Many data have been accumulated which suggest that elevated plasma levels of free fatty acids may play a sinister role in the natural history of myocardial ischaemia. Patients with myocardial infarction have raised plasma levels of free fatty acids during the first 24 h following the onset of symptoms (Kurien and Oliver, 1966) and where plasma free fatty acid levels exceed 1200 mol/l there is a significant rise in the frequency of serious ventricular arrhythmias and an increased risk of dying (Oliver, Kurien and Greenwood, 1968; Gupta *et al.*, 1969).

We, like others (Kjekshus and Mjøs, 1973), have been interested in the possibility that plasma levels of free fatty acids might be causally associated with the degree of structural damage to the myocardium. The model we have used to examine this question is the well-known one of isoprenaline-induced necrosis in the rat heart (Chappel *et al.*, 1959). While of course this cannot be regarded as the homologue of the events leading to transmural myocardial infarction in man or experimental animals, it nevertheless has some attractions. Firstly, since isoprenaline is a sympathomimetic

amine it may well operate within the heart much as endogenous catecholamines do. Secondly, the morphological changes which it produces in the rat heart mimic in some measure those produced in the human by generalized poor coronary artery perfusion (Woolf *et al.*, 1976).

We have shown previously (Woolf *et al.*, 1976) that isoprenaline-induced myocardial necrosis in the rat can be quantitated objectively on the basis of a simple enzyme histochemical technique. This paper records our experience of the effect of metabolic intervention in the events of isoprenaline-induced necrosis by the prior administration of an anti-lipolytic agent—a fluorinated nicotinic acid kindly made available to us through the courtesy of Astra Läkemedel, Södertalje, Sweden.

MATERIALS AND METHODS

One hundred and sixty-four male albino rats weighing approximately 250 g were used in this study. All were of the Charles River CD strain (Sprague–Dawley-derived).

The amine used was isoprenaline bitartrate (a 99% pure laevoisomer manufactured by Aldrich Chemical Co. Inc.). The amine was dissolved in distilled water and was given by s.c. injection into the right flank. The solution used contained 10 mg/ml and a standard dose of 20 mg/kg body weight was given.

Blood from 44 animals was used to determine the free fatty acid levels. Groups of rats were stupefied by placing them in a CO_2-rich atmosphere and bled by heart puncture. The plasma-free fatty acids were measured by the method of Laurell and Tibbling (1964). In this method the free fatty acids are extracted into a chloroform–heptane–methanol mixture and their copper soaps formed by the addition of a copper–tri-ethanolamine solution. The copper content is then measured colorimetrically following reaction with diphenyl carbazide. The validity of this assay was checked by comparison with a gas–liquid chromatography procedure.

In half the animals the isoprenaline injections were preceded by the administration of the nicotinic acid analogue either as the salt (SAB 515) or the active metabolite SAB 509 (SAB 515) given s.c. at a dose of 500 mg/kg body weight 30 min. before the isoprenaline.

One hundred and twenty of the rats were allowed to survive for 72 h after treatment and then killed by exposing them to carbon dioxide. The hearts were removed while still beating and immediately arrested in 0·15 M KCl at 4°. A block taken through the apices of both ventricles was then snap-frozen in isopentane at −70°. Frozen sections were prepared from these blocks as described previously (Woolf *et al.*, 1976). These were then treated to demonstrate succinate dehydrogenase using nitro-blue tetrazolium as the hydrogen acceptor (Chayen *et al.*, 1969). The specificity of this method was shown by the complete inhibition of formazan production in sections incubated in a medium to which malonate had been added. Necrotic muscle fibres showed no evidence of formazan deposition, thus allowing easy distinction between viable and necrotic muscle.

The degree of myocardial muscle loss in each section was measured using an automated flying spot microscope (Eccles *et al.*, 1976). This instrument, a special-purpose computer attached to a microscope with a small PDP 8-E) general purpose computer on line, examines the field of view against a square grid of 256 × 256 points and extracts the data from this field under control of the programme with which it has been loaded. In this study it was used essentially as a scanning microphotometer. At a magnification of × 400 the separation of the points on the grid (*i.e.* the spacing at which the field was sampled) was 0·7 μm. At each grid point the light transmission was measured in green light ($\lambda = 510$ nm) with an accuracy slightly better than 2%. Three thresholds were set by means of which the value of light transmission allowed a point to be categorized as solid tissue showing the presence of formazan (viable muscle), solid tissue devoid of formazan (necrotic areas) or empty spaces (ventricular cavity and vessels). Since each point is representative of its immediate area in the field of view, a count of points lying within the ranges determined by the threshold values, provides a measure of the area of the field occupied by the specified categories. The programme gave just these counts as output. The whole of each section was measured in this way and the procedure gives results which are reproducible with a coefficient of variation of 5·3% (Lenciewicz, Davies and Rosen, 1972). The results in each section are expressed as a percentage of necrotic muscle fibres/viable fibres + necrotic fibres (Woolf *et al.*, 1976).

RESULTS

In the animals pre-dosed with nicotinic acid the percentage loss of heart muscle fibres ranged from 5 to 25% with a

mean of 18%. In those rats where the isoprenaline had not been preceded by an anti-lipolytic agent, the percentages ranged from 16 to 35 with a mean of 27·9%. Statistical analysis shows the differences in the raw data to be significant ($P < 0.001$) (Fig. 1).

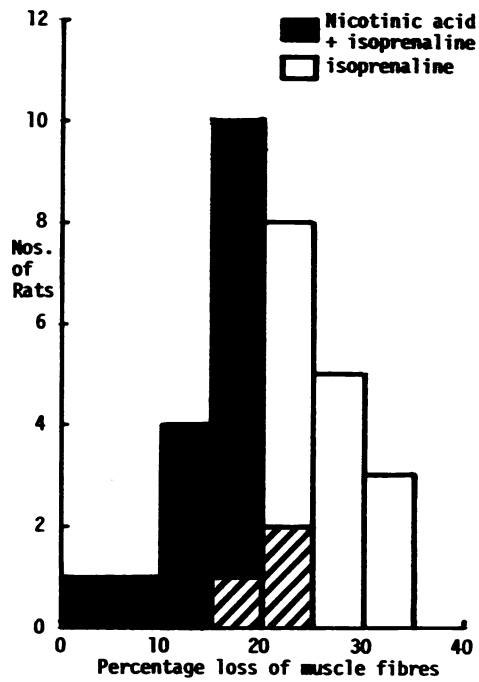

FIG. 1.—Effect of pre-dosing with nicotinic acid.

The animals pre-dosed with the hydrochloride of 5-fluoro-3-pyridylmethanol (fluoro-nicotinic acid) showed loss of muscle fibres ranging from 8 to 28% (mean 16·7). In the control experiments carried out at the same time the degree of damage was greater (range 10–32%, mean 23·9%) (Fig. 2).

When the active compound 5-fluoro-nicotinic acid was administered the greatest protective effect was seen. These animals showed damage to the myocardium ranging from 5 to 25%, mean 15·8%. The control animals which were given ISP only showed loss of fibres ranging from 14 to 36%, mean 27·63%.

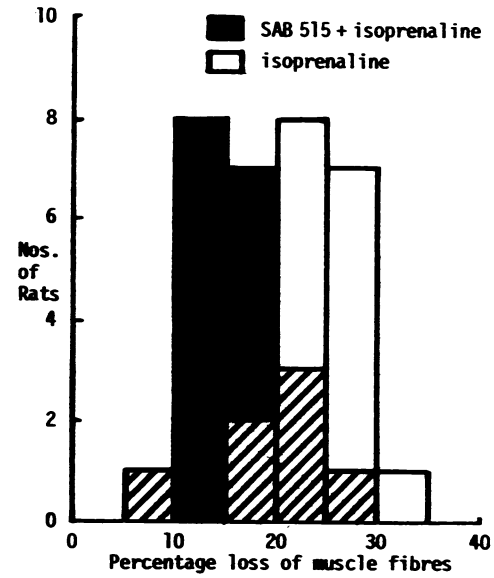

FIG. 2.—Effect of pre-dosing with SAB 515.

The differences below, animals given ISP only and those pre-dosed with either fluoro-nicotinic or 5-fluoro-3-pyridylmethanol were significant ($P < 0.001$) (Figs. 2 and 3).

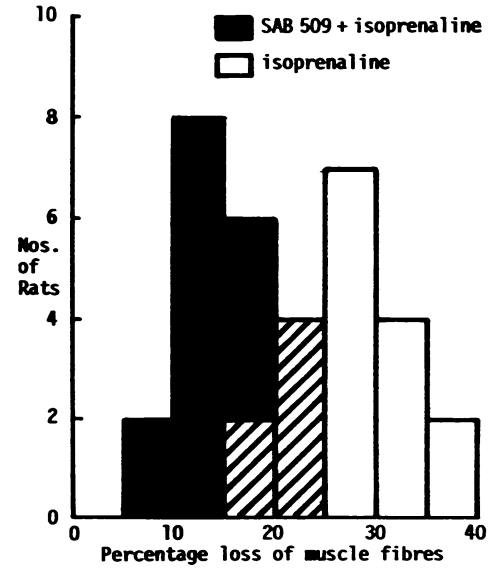

FIG. 3.—Effect of pre-dosing with SAB 509.

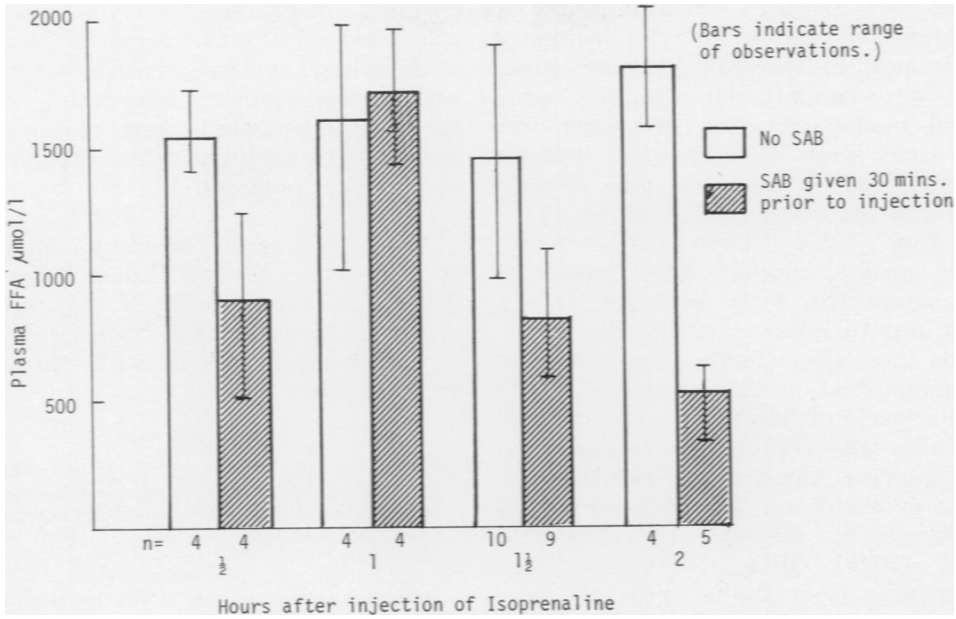

FIG. 4.—Effect of SAB 515 on plasma FFA levels after isoprenaline.

The effect on ISP induced changes in plasma free fatty acid levels

Thirty min. after injection of the ISP with plasma FFA in the pre-dosed group were 900 μmol/l while those in the group receiving no anti-lipolytic were 1500 μmol/l (Fig. 4). At 1 h there was little difference; indeed the pre-dosed group had slightly higher levels than the group receiving ISP only.

However, at 1 h and 2 h after injection of the isoprenaline marked differences were present, the pre-dosing with 5-fluoro-nicotinic acid being associated with much lower plasma levels of FFA. This was particularly striking at the 2-h point, where the levels in the animals given the anti-lypolytic agent were only about one third of the mean in those given ISP only.

DISCUSSION

These results show that pre-dosing with fluorinated nicotinic acid considerably reduces the degree of damage produced in the rat myocardium by a standard dose of isoprenaline bitartrate. In addition at 3 of the 4 points in time following the administration of isoprenaline, at which we have measured the plasma levels of free fatty acids in the rat, the prior administration of 5-fluoro-nicotinic acid was associated with significantly lower plasma levels of free fatty acid.

Despite the apparent causal association between these data it would seem appropriate to examine briefly, but critically, the possible mechanisms by which the protective effect of the nicotinic acid analogue is being exerted.

In an entirely different experimental frame of reference from that provided by isoprenaline cardiotoxicity, Kjekshus and Mjøs (1973) have shown that pre-treatment with the anti-lipolytic agent β pyridylcarbinol reduces the degree of ischaemic injury produced in the dog heart by occlusion of the anterior descending branch of the left coronary artery in an "open-chest" preparation. The measure used in these studies was the

degree of ST segment elevation following temporary occlusion of the coronary artery and no morphological investigations were undertaken. In the same animal model, elevation of plasma free fatty acid levels following i.v. infusion of a triglyceride emulsion plus heparin also increased average ST segment elevation from $1{\cdot}2 \pm 0{\cdot}7$ to $2{\cdot}2 \pm 0{\cdot}8$ mV. These results support the hypothesis that excess free fatty acids produce a direct harmful effect on myocardial fibres.

This view gains further support from the demonstration that increases in the plasma levels of free fatty acids induced by similar triglyceride infusions in normal dogs increase the oxygen requirements of the myocardium, this being unrelated to changes in myocardial performance (Mjøs, 1971*a*). Mjøs (1971*b*) has also shown that prior dosing with an antilipolytic agent in dogs subsequently given isoprenaline inhibits much of the expected increase in myocardial oxygen requirement without affecting the inotropic properties of this amine.

The possibility that alternative or additional mechanisms may operate in the protection conferred by fluorinated nicotinic acid against the cardiotoxicity of isoprenaline also merits examination, since Fleckenstein *et al.* (1973) have adduced evidence that the key event in isoprenaline-induced myocardial necrosis is calcium overlead of the myocardial fibres leading to severe high-energy phosphate deficiency. In a heart muscle cell preparation, Powell and Twist (1976) have shown that the introduction of isoprenaline into the incubating medium is followed by induction of the adenylcyclase system and an increase in cAMP. Nicotinic acid decreases available cAMP in adipose tissue and by this means decreases lipolysis (Butcher, Baird and Sutherland, 1968). It is possible, therefore, that the protective effect of fluorinated nicotinic acid which we have demonstrated may not be due solely to a relative lowering of plasma free fatty acid levels, but may be mediated additionally by a direct effect on the myocardial cell associated with some degree of blocking of the adenyl cyclase induction associated with administration of isoprenaline. Studies on disaggregated heart muscle cells designed to examine this process are currently in progress.

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