Inhibition of Rat Arterial Smooth Muscle Cell Proliferation by Heparin

In Vivo Studies with Anticoagulant and Nonanticoagulant Heparin

JOHN R. GUYTON, ROBERT D. ROSENBERG, ALEXANDER W. CLOWES, AND MORRIS J. KARNOVSKY

SUMMARY  Heparin inhibits the proliferation of intimal smooth muscle cells which occurs after denudation of endothelium by air-drying injury in the rat carotid artery. We determined (1) whether the antiproliferative effect of heparin is secondary to effects on platelet adherence to subendothelium or endothelial regeneration and (2) whether the antiproliferative and anticoagulant activities of heparin are related. Morphometric observations by scanning electron microscopy showed that heparin did not alter platelet adherence 5 days after arterial injury and had little or no effect on endothelial regeneration at 5 and 10 days. To study the relationship between the antiproliferative and anticoagulant effects, we fractionated heparin by affinity chromatography on antithrombin-Sepharose into purified anticoagulant and nonanticoagulant fractions. These heparin fractions were administered to rats in doses which were equivalent either in terms of anticoagulant activity or in terms of mass to the dosage of unfractionated heparin known to inhibit myointimal growth. Additionally, some rats received nonanticoagulant heparin at a dose which was greater in terms of mass than the highest dose of unfractionated heparin which could be administered without inducing fatal hemorrhage. Inhibition of myointimal growth, determined by morphometric analysis of total plaque volume 2 weeks after arterial injury, correlated with total mass of heparin administered but not with anticoagulant activity. Non-anticoagulant heparin given at high dose caused 77% inhibition of myointimal growth ($P = 0.02$ vs. controls). Heparin inhibition of arterial smooth muscle cell proliferation does not appear to be mediated either by effects on other cells at the level of the arterial wall or by antithrombin. This study should direct attention toward a potential growth regulatory role for arterial glycosaminoglycans.


IN A RECENT study on intimal smooth muscle cell proliferation in the rat carotid artery denuded of endothelium, we reported that intravenous heparin in doses large enough to cause continuous anticoagulation markedly inhibited myointimal proliferation (Clowes and Karnovsky, 1977). Several hypotheses for the mechanism of this antiproliferative effect of heparin have been considered. First, heparin may have little direct effect on smooth muscle cells in vivo, but may change platelet function (Zucker, 1974) or endothelial regeneration in a manner such that smooth muscle cell proliferation is secondarily inhibited. Second, activation of clotting factors may be necessary for smooth muscle cell proliferation, either directly, as in thrombin stimulation of fibroblast division in tissue culture (Chen and Buchanan, 1975), or indirectly, since thrombin evolved at an injured vessel wall could potentiate platelet aggregation and release of platelet-derived growth factors (Ross et al., 1974). The discovery in the laboratory of one of us (R. D. Rosenberg) that heparin can be separated into anticoagulant and nonanticoagulant fractions (Lam et al., 1976) has made possible investigations of the hypothesized link between humoral clotting and smooth muscle cell proliferation in vivo as well as in vitro. Finally, heparin may interfere with the action of growth factors other than clotting factors, or it may act directly on the cell to inhibit division.

In this paper we report in vivo studies of the mechanism of the antiproliferative action of heparin on arterial smooth muscle cells. It is shown, first, that this effect is unlikely to be caused by antecedent changes in endothelial regeneration or platelet function. Second, because nonanticoagulant heparin is capable of inhibiting myointimal proliferation,
the antiproliferative effect probably is not related to changes in the humoral clotting system.

Methods
Preparation of Experimental Animals

We studied 69 male Sprague-Dawley rats (CD strain, Charles River Breeding Laboratories) weighing between 250 and 320 g. Arterial air-drying injury was induced using the technique of Fishman et al. (1975) modified with a higher air flow rate and longer drying time to ensure complete endothelial denudation. Briefly, the rat was anesthetized with intraperitoneal pentobarbital (Nembutal, Abbott Laboratories, 50 mg/kg body weight) and the right common carotid artery was exposed and ligated at two points 1.5 cm apart. A 30-gauge hypodermic needle was inserted into the proximal end of the segment. An exit hole was punctured at the distal end of the segment either by passing the same needle down the lumen and out through the wall or by puncturing with an additional needle from outside. After the lumen had been rinsed with phosphate-buffered saline, dry (compressed) air was allowed to flow through the segment for 3.5 minutes at a rate of 50-60 ml/min. Ligatures then were removed and hemostasis was obtained by pressure.

Within minutes to a few hours after reestablishment of blood flow in the arterial segment, desicated endothelial cells detach from the arterial wall and are swept away. To monitor the completeness of endothelial denudation between the ligature sites, perfusion silver staining (Fishman et al., 1975) was performed 24 hours after operation in nine rats taken from various cohorts during the course of the study.

The intravenous infusion technique was the same as that described previously (Clowes and Karnovsky, 1977), except that the swivel connector was found to be unnecessary. A Silastic catheter was placed in the left jugular vein and passed through the skin at the back of the neck beneath a shoulder saddle, where it connected to an external catheter of Tygon tubing. The external catheter was protected by a stainless steel flexible coil which was anchored 14 or more inches above the animal. The animal’s cage was turned daily to relieve any torque of the animal’s net rotational movement. A syringe pump delivered fluid at a rate of 0.91 ml/min. Ringer’s lactate (Abbott) was infused into control rats and served as diluent for the heparin fractions.

Preparation of Experimental Animals

Human thrombin and human antithrombin were isolated in physically homogeneous form by methods previously reported (Rosenberg and Damus, 1973).

The anticoagulant potency of mucopolysaccharide fractions was estimated by quantifying their ability to accelerate the interaction of antithrombin with thrombin and comparing the extent of enzyme neutralization to that attained with a heparin standard of known USP potency (Lam et al., 1976).

The concentration of antithrombin was determined by absorbance measurements at 280 nm assuming an extinction coefficient of 6.5. Mucopolysaccharide concentrations were estimated colorimetrically by assay of uronic acid at 530 nm according to the carbozole method of Bitter and Muir (1962). The relationship between this parameter and the dry weight of heparin fractions was determined experimentally.

A single lot of commercial heparin (Upjohn, lot 082ED) was used to prepare the heparin fractions. Heparin was mixed with a 2-fold molar excess of antithrombin to form complexes in a buffer consisting of 0.15 M NaCl in 0.01 M Tris-HCl at pH 7.5 and 24°C. Thereafter, heparin bound to inhibitor as well as uncomplexed mucopolysaccharide were isolated free of antithrombin by techniques analogous to those reported previously (Rosenberg et al., 1978; Jordan et al., 1979). The heparin that bound to antithrombin, called purified anticoagulant heparin, exhibited a specific anticoagulant activity of 317-352 USP units/mg. The mucopolysaccharide that did not complex with antithrombin, termed nonanticoagulant heparin, had a specific anticoagulant activity of 17-18 USP units/mg.

Chemical differences between anticoagulant and nonanticoagulant heparin are subtle. Analysis of low molecular weight fractions (~6000 daltons) has demonstrated that anticoagulant heparin contains 1.1 more residues of glucuronic acid and 1.5 fewer residues of N-sulfated glucosamine than nonanticoagulant heparin (Rosenberg et al., 1978). Recent studies have demonstrated the unique presence in anticoagulant heparin of a tetrasaccharide sequence with the unusual features of nonsulfated iduronic and glucuronic acid residues and an N-acetylated glucosamine (Rosenberg and Lam, 1979).

Platelet Deposition and Endothelial Regeneration

The inner surface of the right carotid artery was studied by scanning electron microscopy at 5 and 10 days after air-drying injury in 34 rats. Arteries from 10 heparin-treated and 9 control rats were fixed at 5 days, and from 9 heparin-treated and 6 control rats were fixed at 10 days. Only unfracti-
for unfractionated heparin shown in Table 1. Two commercial brands of porcine intestinal heparin (Elkin-Sinn and Upjohn) were used at various times, both of which were equally effective in inhibiting rat arterial smooth muscle cell proliferation in vitro (data not shown).

After blood had been drawn for Lee-White clotting times, hematocrits, and platelet counts (hematocytometer method), rats were fixed by retrograde perfusion via the abdominal aorta at 120 mm Hg pressure with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer.

Excised tissues, including right and left common carotid arteries, were further fixed by immersion in 2% paraformaldehyde and 2.5% glutaraldehyde for 2-4 hours. Arteries were postfixed in osmium tetroxide and dehydrated using alcohols. They were sectioned at the midpoint, cut longitudinally, and were treated as described earlier. Carotids were sectioned midway between the original needle puncture sites and embedded in Epon 812. Two-micron sections were cut perpendicular to the vessel axis at 500-μm intervals to a depth of 5 mm in each half of the original carotid segment, thus yielding 22 sections for plaque volume analysis.

To quantify platelet adherence to the arterial wall at 5 days, three fields from each artery selected at low magnification were photographed at 3000×. The photographs were coded and shuffled, and platelets were counted visually, marking those counted on a plastic overlay. Eight photographs were counted twice, showing good reproducibility, with an intraclass correlation coefficient of 1.00. The total weight of the intimal plaque volume was contained within ± 1% of the outlines of intima, media, and media were traced, and the paper was cut and projected at approximately 200-fold magnification onto bond quality typing paper which varied in weight per unit area by ± 1%. The outlines of intima and media were traced, and the paper was cut and weighed. Repeated area determinations for individual samples of intima and media from 20 sections showed good reproducibility, with an intraclass correlation coefficient of 1.00. The total weight of

### Table 1

<table>
<thead>
<tr>
<th>Heparin fraction infused</th>
<th>Dose schedule</th>
<th>Days post-op</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (7)*</td>
<td>1</td>
<td>2-4</td>
</tr>
<tr>
<td>Unfractionated (UF) (7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anticoagulant (AC) (4)</td>
<td>0.39</td>
<td>0.49</td>
</tr>
<tr>
<td>Nonanticoagulant (NAC1)</td>
<td>0.39</td>
<td>0.49</td>
</tr>
<tr>
<td>Nonanticoagulant (NAC2)</td>
<td>0.78</td>
<td>0.78</td>
</tr>
</tbody>
</table>

* Numbers in parentheses = numbers of rats.
† The dosage schedule for the group receiving unfractionated heparin is given in terms of both anticoagulant activity and mass.
tracings of intima was divided by the total weight of tracings of media to give an index of plaque volume in each animal.

**Autopsies**

After perfusion fixation, viscera in all rats were inspected grossly for abnormality. In 21 representative animals, paraffin sections of various organs were stained with hematoxylin and eosin and with alcian blue and neutral red.

**Statistical Methods**

Reproducibility of morphometric technique was shown by the intraclass correlation coefficient, which yields values near 1.00 when the variance among replicated measurements is small compared to the variance among measurements on different specimens (Snedecor and Cochran, 1956). To demonstrate the lack of difference between heparinized and control groups in endothelial regeneration and platelet adherence, Student's t-test with confidence intervals for the differences between means was used. Analysis of variance was used to assess differences in plaque volume, with data from each animal transformed to the fourth root to give comparable variances within groups. Transformation to a root was more suitable than either the use of untransformed data or log transformation, because the variance could be expected to increase with increasing values, yet values near zero (i.e., almost no migration of smooth muscle cells into the intima) were possible and did occur. Scheffe analysis was chosen for a posteriori comparison of results, because it corrects for multiplicity of comparisons.

**Figure 1** Scanning electron micrograph showing platelets adherent to arterial wall 5 days after air-drying injury and 4 days after institution of heparin therapy, in a healthy animal without bleeding. Bar = 5 μm. 5800x.
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(more conservatively than other methods) and at the same time allows combinations of groups to be compared (Brownlee, 1965).

Results

Completeness of Endothelial Denudation

Silver stains performed the day after air-drying carotid injury showed 98-100% endothelial denudation within the ligature marks in all nine rats. Three rats showed 100% denudation; in the other six a small tag of endothelium remained intact at the end of the segment next to a ligature mark, where it presumably was protected from drying by moisture trapped in crevices or beneath the needle tip.

Weight Gain, Blood Findings, Histology

There were no significant differences among experimental and control groups for weight gain (average was 21 g), platelet counts, or hematocrits. Routine hematoxylin and eosin staining of various organs showed no unusual findings. Alcian blue applied to the paraffin sections stained mucus, bone marrow cell granules, aortic media in all animals, and bony matrix variably. In one rat given high-dose nonanticoagulant heparin, alcian blue staining was seen in scattered large cells in the spleen.

Platelet Adherence

Figure 1 is a typical scanning electron micrograph of adherent platelets, from which visual counting was performed. Six rats which remained healthy after operation and heparinization gave results that were sharply different from those of three rats which bled massively and were moribund at the time of fixation. The former group showed a mean (± SEM) of 199,000 ± 14,000 platelets per square millimeter adherent to the arterial wall, which is not significantly different from the mean of 216,000 ± 13,000 found in unheparinized controls (Fig. 2). The three rats which bled had a range of platelet adherence from 10,000 to 55,000 per square millimeter (not shown in Fig. 2). This latter group could not be considered representative of rats which were submitted to studies lasting longer than 5 days, because in the longer studies rats which showed neck swelling and pallor at 5 days always died when the heparin dose was increased.

Endothelial Regeneration

A typical view of endothelium regenerating as a continuous sheet of cells is shown in Figure 3. Heparin appeared to have little effect on the fraction of denuded surface reinvested by endothelium at 5 and 10 days (Fig. 4). Heparin-treated animals had slightly less endothelial regeneration at 5 days, but the differences between heparin-treated and control groups were not significant at either time period.

Anticoagulant Activity and Myointimal Thickening

Clotting times were prolonged to approximately 2.5 times control values after administration of either unfractionated heparin or the anticoagulant fraction (Fig. 5). Two of the eight rats given nonanticoagulant heparin had clotting times slightly outside the normal range (7.5 and 7.8 minutes vs. a range of 3.7 to 5.8 minutes in control rats), but the mean log clotting times of nonanticoagulant heparin groups were not significantly different from controls.

Figure 6 shows light micrographs of myointimal thickening in a control rat and a rat given nonanticoagulant heparin. Determinations of intima-media volume ratios (Fig. 7 A and B) clearly showed inhibition of myointimal thickening in the high-dose nonanticoagulant heparin group (77% reduction in plaque size, P = 0.02 vs. controls) as well as in the unfractionated heparin group (64% reduction in plaque size, P = 0.02 vs. controls). There was also a significant difference between the group given the anticoagulant heparin fraction and the high-dose nonanticoagulant heparin group (P = 0.04). Scheffé analysis also allows a posteriori comparisons of combinations of groups; results are given in the legend to Figure 7.

Figure 7a presents data on intima-media volume ratios in individual arteries, plotted against the total cumulative dose of heparin in terms of mass. A negative correlation between mass dose of heparin and plaque growth is evident on visual inspection.

As shown in Figure 7b, the unfractionated heparin group tended to show greater inhibition of
plaque growth than the nonanticoagulant heparin group given the same mass dose (NAC1) and less inhibition than the group given a higher dose of nonanticoagulant heparin (NAC2). However, these trends did not reach statistical significance.

**Discussion**

Our results confirm earlier work (Clowes and Karnovsky, 1977), in demonstrating an inhibitory effect of heparin on intimal smooth muscle cell proliferation, which can account for as much as 77% inhibition of growth in terms of plaque volume. With the same progressive dosage regime used to demonstrate this effect, heparin had little or no effect on platelet adherence or endothelial regeneration. Experiments with heparin fractions differing in their binding to antithrombin showed that the antiproliferative effect of heparin is not related to its anticoagulant activity.

**Role of Platelets**

It is likely that platelets did play a role in initiating smooth muscle cell proliferation in this study, as has been demonstrated in other models of arterial endothelial denudation (Moore et al., 1976; Friedman et al., 1977). Heparin conceivably could interfere with platelet-mediated activation of smooth muscle cells either by inhibiting platelet attachment to subendothelium and granule release or by preventing platelet release products from exerting their effects on the smooth muscle cells. There are several reasons to believe that the antiproliferative effect of heparin in vivo is not mediated through the former mechanism—i.e., de-
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FIGURE 4 Endothelial regeneration in control and heparin-treated rats assessed by scanning electron microscopy 5 and 10 days after air-drying injury to the right carotid artery. Mean ± SEM are shown. Numbers of rats in each group are in parentheses. Means for the heparin-treated groups (XH) and control (xc) and 95% confidence intervals (C.I.) for the difference between means were as follows: At 5 days, XH = 0.43, xc = 0.54, C.I. = -0.28 to +0.08. At 10 days, XH = 0.72, xc = 0.73. C.I. = -0.22 to +0.21.

FIGURE 5 Whole blood clotting times after 2 weeks of continuous infusion of various forms of heparin. The scale is logarithmic, and geometric means ± SEM are shown. Groups are as in Table 1. UF = unfractionated heparin, AC = anticoagulant heparin, NAC1 = nonanticoagulant heparin at equivalent mass dose, NAC2 = high-dose nonanticoagulant heparin. Using Student's t-test with correction for unequal variances, a significant difference from control was found for UF (P = 0.003), but not for AC (P = 0.07) or any other group. Filled circles represent anticoagulated groups; unfilled circles represent nonanticoagulated groups.

FIGURE 6 Appearance of intimal plaque near the mid-point of injured arterial segment. A: Control rat. Arrow indicates internal elastic lamina. B: Rat given high-dose nonanticoagulant heparin. Arrow indicates internal elastic lamina. Toluidine blue, 490X; bar = 20 μm.

rangement of platelet function. First, heparin inhibits smooth muscle cell growth in vitro in the presence of platelet products (R.L. Hoover, R.D. Rosenberg, and M.J. Karnovsky, unpublished observation). Second, heparin was not administered in this study until 24 hours after carotid air-drying injury, with the exception of the high-dose nonanticoagulant heparin group. Endothelial desquamation and platelet adherence are actually seen within minutes after air-drying injury to the rat carotid artery and reestablishment of blood flow. Complete coverage of the denuded surface and degranulation of platelets follow within a few hours (unpublished observations). Subsequent turnover of platelets at the arterial surface probably is relatively slow, as shown by Groves and co-workers (1979) after balloon catheter endothelial denudation in the rabbit. Friedman (1977) has reported that severe thrombocytopenia initiated 1 day after endothelial denudation in the rabbit aorta does not inhibit the smooth muscle cell proliferative response. This fact suggests that platelets may trigger the smooth muscle cell response, but are not necessary to sustain it. Together, all these observations suggest that the major antiproliferative effect of heparin is likely to occur at some step subsequent to platelet degranulation.

In the absence of massive hemorrhage, heparin did not appear to affect platelet adherence to the arterial wall, observed 5 days after endothelial denudation and 4 days after the onset of heparin administration. This is in accord with the finding of Essien and co-workers (1978) that heparin did not
carotid injury model had suggested that, if a certain
AC vs. NAC2, P = 0.04; control vs. NACl + NAC2, P =
0.03; AC vs. UF + NAC1 + NAC2, P = 0.01; control +
AC vs. UF + NAC1 + NAC2, P = 0.002.

Endothelial Regeneration
Preliminary experience with the air-drying rat carotid injury model had suggested that, if a certain
small amount of viable endothelium, perhaps 10-
20%, remained within the ligature sites after the
injury, smooth muscle cell proliferation would be
inhibited markedly, presumably because of rapid
re-endothelialization. This is the reason that silver
stains 1 day after injury were performed repeatedly
during the course of the study. It also was necessary
to determine whether heparin might accelerate en-
dothelial regrowth and thereby inhibit smooth mus-
cle cell proliferation indirectly. Figure 4 shows that
this clearly was not the case.

Effectiveness of Nonanticoagulant Heparin
The most important conclusion of this study is
that inhibition of smooth muscle cell proliferation
by heparin does not require anticoagulant activity.
Our in vitro data support this conclusion, and it is
also consistent with the studies of Lippman and
Mathews (1977) on L-M cells in tissue culture. They
demonstrated a lack of correlation between antico-
agulant and antiproliferative effects of various types
of heparin. It may be inferred that thrombin, de-
spite its potent mitogenic effect in fibroblast tissue
culture (Chen and Buchanan, 1975), probably does
not play a major or necessary role in the smooth
muscle cell proliferative response. A modulating
role for thrombin is not ruled out by the data,
particularly in view of the trend toward a greater
nonproliferative effect of unfractionated heparin as
opposed to nonanticoagulant heparin at the same
mass dose (NAC1 group in Fig. 7).

The data from this in vivo study do not answer
the question of whether the purified anticoagulant
heparin fraction might inhibit myointimal prolifer-
ation as effectively as the nonanticoagulant heparin
fraction. Recent in vitro work in our laboratory
suggests that, in fact, the two fractions have equal
antiproliferative potency (R.L. Hoover, R.D. Ro-
senberg, and M.J. Karnovsky, unpublished obser-
vation). The maximum mass dose of purified anti-
coagulant heparin that could be administered safely
in vivo appears to be below the threshold necessary
for a distinct antiproliferative effect, as shown in
Figure 7b.

Although heparin is a mast cell product and has
not been found in the intima or media of arteries,
other glycosaminoglycans, including the closely re-
lated compound, heparan sulfate, are present at high
concentrations (Smith, 1973). Some of these glyco-
saminoglycans may be inhibitory for smooth muscle
cell growth, as suggested by Eisenstein and col-
leagues (1979). Wasteson and co-workers (1977)
have demonstrated that platelets contain an en-
zyme that specifically degrades heparin and he-
paran sulfate. It is possible that this platelet enzyme
might act as a growth factor in concert with the
well-known small cationic polypeptide growth fac-
tor (Ross and Vogel, 1978). Whatever the initial
stimulus for medial smooth muscle cell activation,
we may speculate that those cells which migrate to
the intima might be exposed to an environment
affect in vitro platelet adherence to rapidly isolated
aortic subendothelium.
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which lacks the usual inhibitory influence of certain medial glycosaminoglycans on cell division and therefore might continue to proliferate for some time after the initial stimulus (perhaps platelet factors) has subsided. Exogenous heparin administration in our study may have acted to restore the physiological inhibitory influence.

An alternative hypothesis for the action of heparin, that it may bind the cationic platelet-derived growth factor before it can contact smooth muscle cells, is considered doubtful. For reasons discussed above, it seems likely that the greatest quantity of platelet-derived growth factor would have reached the surface of smooth muscle cells during the first 24 hours, before heparin was given in most of our experiments. Also, in vitro work suggests that contact between heparin and the cell surface is a critical step in the antiproliferative effect (R.L. Hoover, R.D. Rosenberg, and M.J. Karnovsky, unpublished observation).

Possible Effects of Heparin on Atherosclerosis and Other Conditions

Studies on the inhibition of dietary atherosclerosis by heparin or heparin-like compounds have yielded variable results (Hess, 1964). Those with positive results have generally regarded plasma lipid changes due to heparin as the ameliorating factor in the atherosclerotic process. Interestingly, two recent studies with positive results [Besterman (1970) and Grossman et al. (1971)] utilized heparin-like compounds chosen for their lack of anticoagulant activity—a sulfated polysaccharide from seaweed and a heparan sulfate by-product from the commercial manufacture of beef lung heparin, respectively.

It would be dubious to propose nonanticoagulant heparin as a treatment for atherosclerosis. In certain clinical circumstances, however, intimal smooth muscle cell proliferation may occur much more rapidly than in ordinary atherosclerosis and thus be amenable to pharmacological inhibition. This situation may occur in some patients after saphenous vein coronary artery bypass grafting (Kern et al., 1972; Lawrie et al., 1976) or after arterial embolectomy by Fogarty balloon catheter (Chidi and DePalma, 1978).

This study suggests that heparin inhibition of arterial smooth muscle cell proliferation occurs through a direct process and is not mediated by effects on other cells or by antithrombin. Thus the antiproliferative effect of heparin should direct attention toward a potential growth regulatory role for arterial glycosaminoglycans, but not toward such a role for the humoral clotting system.

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Comparison of the Effects of Regional Ischemia, Hypoxia, Hyperkalemia, and Acidosis on Intracellular and Extracellular Potentials and Metabolism in the Isolated Porcine Heart

HERVÉ MORENA, MICHEL J. JANSE, JAN W.T. FIOLET, WILLEM J.G. KRIEGER, HARRY CRIJNS, AND D. DURER

SUMMARY DC electrograms and transmembrane potentials were recorded from isolated perfused pig hearts. Regional ischemia was produced by clamping the left anterior descending artery (LAD), and after a reperfusion period, regional hypoxia and/or hyperkalemia was produced by perfusing the LAD with hypoxic, glucose-free solutions (with or without acidification, or high K+) or with normoxic high K+ solutions. In transmural biopsies, nucleotides, lactate, and K+ were determined. During ischemia, resting potential decreases (T-Q depression), action potential amplitude and upstroke velocity decrease, and local activation is markedly delayed (S-T elevation, late intrinsic deflection, high R wave). A high K+ concentration, up to 13 mM, decreases resting potential (T-Q depression) and shortens the action potential (positive T wave) but has minor effect on amplitude (no S-T elevation) and activation (no delay). Hypoxia (P O2 = 7 mm Hg, no glucose) causes a moderate decrease in resting potential, marked action potential shortening, and some loss of amplitude but no or only minor delay in activation (slight T-Q depression and S-T elevation, positive T waves). Acidic perfusate does not influence changes in transmembrane potential during hypoxia. Potentials of similar configuration to those seen during ischemia could be obtained by LAD perfusion with hypoxic, glucose-free, high K+ (10 mM), acidic (pH 6.8) solutions. Most surprising was improvement of potentials after 20 minutes of perfusion, like that seen during maintained LAD occlusion. The time course of metabolic changes was the same in hypoxia and ischemia. Results indicate (1) that there is no direct relationship between metabolic and electrical changes, (2) that electrical changes during ischemia are caused by a combination of lack of perfusion (hypoxia, no substrate) and lack of washout (hyperkalemia, acidosis), and (3) that action potentials of ischemic cells are more "depressed" than those of normoxic cells, at similar reduced levels of resting membrane potential.


IT IS known that within minutes after coronary artery occlusion the resting membrane potential of ventricular cells decreases and the amplitude and duration of the transmembrane action potential diminish (Kléber et al., 1978). These changes may be due on the one hand to the sudden inavailability of oxygen and substrate, and on the other hand to the lack of washout and the resulting accumulation of substances including potassium in the extracellular space. It is known that both time course and nature of electrophysiological changes during ischemia are different from those during hypoxia (Trautwein et al., 1954; Kardesch et al., 1958; Bagdonas et al., 1961; McDonald and MacLeod, 1973; Kléber et al., 1978), and many authors therefore have emphasized the role of the accumulation of K+ in explaining the electrical changes during is-
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