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Acetylsalicylic acid enhances arrhythmogeneity in a model of local ischemia of isolated rabbit hearts

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Abstract

Acetylsalicylic acid often is used in the treatment and prophylaxis of regional myocardial ischemia and infarction. However, only little is known about its electrophysiological effects and on possible proarrhythmic effects of the drug. Thus, the aim of this study was to evaluate the electrophysiological effects of acetylsalicylic acid in normal isolated saline perfused rabbit hearts and in hearts submitted to regional ischemia. Isolated saline perfused rabbit hearts were treated with increasing concentrations of acetylsalicylic acid (0.05, 0.1, 0.5 and 1 μM). The epicardial activation and repolarisation process were analysed using an epicardial mapping (256 unipolar leads). Activation and repolarisation time were determined for each electrode from which data the 'breakthrough-points' of epicardial activation were determined. At each electrode an activation vector was calculated giving the direction and velocity of the local excitation wave. The similarity of selected heart beats compared to the control was evaluated by determination of the percentage of identical breakthrough-points and of similar vectors (deviation $\leq 5°$). At each electrode the local epicardial action potential duration was assessed as the activation recovery interval and the standard deviation of the epicardial action potential duration (of 256 leads, = dispersion) was determined. In a second series of experiments 30 min regional ischemia was induced by occlusion of the left descendent coronary artery followed by 30 min reperfusion in the absence or presence of 0.5 μM acetylsalicylic acid or 1 μM indomethacin. The degree of ischemia was assessed by the reduction in coronary flow, by the degree of ST-elevation and by the area in which ST-elevation was registered. Under non-ischemic conditions acetylsalicylic acid led to an increase in the epicardial action potential duration (7%), a decrease in the breakthrough-point similarity (by 10%) and vectorfield similarity (by 15%). In control hearts submitted to regional ischemia the similarity of the vectorfields and of the breakthrough-points, as well as the duration of the epicardial action potentials were markedly reduced while the dispersion was greatly increased. In the ischemic region there was a significant ST-deviation from the isoelectrical line. These changes of ST-segments were significantly enhanced by 0.5 μM acetylsalicylic acid, so that in all (7/7) acetylsalicylic acid treated hearts sustained ventricular fibrillation occurred after 20 min ischemia, whereas in the absence of acetylsalicylic acid fibrillation was found in only 2/7 hearts during reperfusion and not during ischemia. 1 μM indomethacin did not cause these changes. In all ischemia/reperfusion series of experiments the reduction in coronary flow and left ventricular pressure by ischemia was of the same degree and we did not observe significant differences in the size of ischemic area. Using ^{14}C-acetylsalicylic acid, an accumulation of acetylsalicylic acid in the ischemic region could be observed. From these results we conclude, that acetylsalicylic acid can induce ventricular fibrillation. Thus, in acute myocardial ischemia, acetylsalicylic acid may have (besides the well known and desired antiaggregatory effects) electrophysiologic side effects which seem to be proarrhythmic in regional ischemia at least in this model. © 1997 Elsevier Science B.V.

Keywords: Arrhythmia; Acetylsalicylic acid; Cardiac ischemia; Cardiac reperfusion; Electrophysiology; Electrocardiology; Epicardial mapping

1. Introduction

Acetylsalicylic acid is widely used in the acute treatment of myocardial infarction as well as in the primary and secondary prevention (Hennekens et al., 1989; Stein et al., 1989; Willich, 1993). Its cyclooxygenase blocking actions prevent platelet aggregation and there is evidence that antiaggregatory treatment with acetylsalicylic acid can significantly reduce cardiovascular mortality in cardiac ischemia and infarction (Hjemdahl-Monsen et al., 1986; Anti-Platelet Trialist Collaboration, 1988; Physicians'

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Health Study Research Group, 1989; Juul-Moller et al., 1992). On the other hand, in a placebo-controlled trial Bourke et al. (1990) reported that the incidence of inducible ventricular tachycardia in patients after myocardial infarction under streptokinase-treatment was significantly reduced but found no comparable beneficial effect of aspirin-treatment.

Very little is known on the cardiac electrophysiological effects of acetylsalicylic acid independent from its antiaggregatory effect in regional ischemia and reperfusion. In some older reports there are hints on action potential prolonging effects of acetylsalicylic acid (Kwiatkowska-Patzer and Herbaczynska-Cedro, 1981). These authors showed that acetylsalicylic acid prolonged the refractory period by 28% in normal cat myocardium when given i.v. in a dosage of 7 mg/kg. Such an increase in refractory period may represent antiarrhythmic activity but on the other hand may be proarrhythmic under certain conditions. Thus, besides this report, it has been shown by others that acetylsalicylic acid may increase arrhythmogeneity in regional ischemia and reperfusion in the rat if compared to thromboxane A2 synthetase inhibitors (Fukuchi et al., 1992), may exert proarrhythmic effects in digoxin treated guinea pig hearts (Kanzik et al., 1991) or cause even a moderate increase in arrhythmia during ischemia/reperfusion in rat hearts (Isensee et al., 1993). However, detailed studies on the electrophysiological effects of acetylsalicylic acid during cardiac ischemia and reperfusion are still missing.

Clinical discussion on possible proarrhythmic effects of acetylsalicylic acid focuses on a possible enhancement of sudden cardiac death under aspirin treatment. In the study of Bourke et al. (1990) the incidence of cardiac death and of Lown IV and V arrhythmia was somewhat higher in the aspirin group as compared to the placebo-treated group. However, this effect was not significant, possibly due to the small number of patients included in that study. In addition, in another study comparing the effectiveness of aspirin and warfarin for the prevention of major vascular events the sudden death rate in the aspirin group was higher (7.7/100 patient-years) compared with the warfarin-group (4.8/100 patient-years) (Chimowitz et al., 1995). Besides this, an increased incidence of sudden death in diabetic patients receiving aspirin and dipyridamole after amputation for gangrene has been reported (Colwell et al., 1986), the basis of which remained unclear. Thus, there has been some concern that acetylsalicylic acid may indeed have some proarrhythmic effect at least under certain conditions.

In order to elucidate the question whether acetylsalicylic acid may prolong action potential duration, whether this may have antiarrhythmic effects or whether acetylsalicylic acid may exert proarrhythmic effects in regional ischemia/reperfusion injury or not, we decided to investigate the influence of acetylsalicylic acid pre-treatment on epicardial action potential duration and activation spreading under non-ischemic conditions and in ischemia/reperfusion injury in comparison to a blockade of cyclooxygenase with indomethacin and to salicylic acid which does not or only very weakly block cyclooxygenase (Chiabrando et al., 1989; Violi et al., 1989; Rizk and Abdel-Rahman, 1994). In order to study these effects independently from the well known beneficial antiaggregatory effects we used a saline perfused rabbit heart model. We decided to investigate the effects of acetylsalicylic acid on cardiac electrophysiology in concentrations as used for antiaggregatory treatment.

Therefore, we chose an acetylsalicylic acid concentration range in the range of the plasma levels reported for the drug, which are in the order of 50 to 1000 ng/ml with maximum concentrations of 24 μg/ml depending on the dosage. 40 to 50% of the drug occurs in plasma as acetylsalicylic acid (i.e. without being des-acetylated; for review see Schrör, 1992). Since in addition FitzGerald et al. (1990) and Clarke et al. (1991) described plasma levels in the order of 50 to 100 ng/ml after administration of a 75 mg slow release acetylsalicylic acid used for antiaggregatory treatment, we decided to investigate the influence of 0.1 to 1.0 μmol/l (corresponding to 18 to 180 ng/ml) acetylsalicylic acid under normal conditions and of 0.5 μmol/l (corresponding to 90 ng/ml) under ischemic conditions.

2. Methods

2.1. Heart-preparation and epicardial mapping

All experiments were performed in accordance with the ethical rules of the Council for International Organisation of Medical Science and the German laws for animal welfare. The method of heart preparation and epicardial potential mapping has been described in more detail previously (Dhein et al., 1993) and will be explained only briefly in the following paragraph.

Male white New Zealand rabbits (conventional, normally fed ad libitum, 1500–1800 g, Fortkamp, Lengerich, FRG) were treated with 1000 IU/kg heparin i.v. 5 min before they were killed by a sharp blow on the neck with subsequent exsanguination. The heart was excised, prepared and perfused according to the Langendorff-technique at constant pressure (70 cm H_2O) with Tyrode's solution of the following composition: Na^+, 161.02; K^+, 5.36; Ca^{2+}, 1.8; Mg^{2+}, 1.05; Cl^-, 147.86; HCO_3^-, 23.8; PO_4^{2-}, 0.42 and glucose 11.1 mmol/l, equilibrated with 95% O_2 and 5% CO_2. The surface temperature of the heart was 37°C. The hearts were connected to a 256 channel mapping system HAL3 (temporal resolution: 4 kHz/channel; amplitude resolution: 0.04 mV, interchannel coupling < -60 dB) as described previously (Dhein et al., 1988). 256 AgCl electrodes were cast in 4 polyester plates (in 8×8 orthogonal matrices with 1 mm interelectrode distance),

which were attached to the hearts surface in an elastic manner, so that they could follow the hearts movements easily without dislocation. The hearts were paced supraventricularly via two Pt-electrodes at the right atrium at their physiological beating rate of 3 Hz.

In a first series of experiments we carried out a time control series over 120 min without any treatment. In order to test the functional and electrophysiological effects of acetylsalicylic acid we perfused acetylsalicylic acid in cumulative concentrations (0.05, 0.1, 0.5 and 1.0 μmol/l; each concentration was given for 15 min).

In another experimental protocol we tested the influence of regional ischemia on the parameters as well as the effect of 0.5 μmol/l acetylsalicylic acid or 1.0 μmol/l indomethacin on the ischemia-induced alterations.

After 1 h of equilibration under standard conditions and after subsequent 15 min pre-treatment with either Tyrode solution (control), 0.5 μmol/l acetylsalicylic acid or 1.0 μmol/l indomethacin a branch of the left anterior descending coronary artery was occluded for 30 min by ligation. Thereafter, the ligation was released and the heart was reperfused for another 30 min. The treatment with the drugs was continued throughout the whole ischemia and reperfusion period in both series.

The degree of ischemia was assessed by: (1) the reduction in coronary flow, (2) the area of the ventricle, in which ST-elevation was observed, (3) the intensity of ST-elevation and finally (4) the reduction in left ventricular pressure.

Epicardial potential mapping was carried out in each experimental phase during periods of constant cycle length of at least 4 min, in order to make it possible to compare the activation patterns (of single heart beats) or their alterations.

In addition, the functional parameters maximum systolic left ventricular pressure, enddiastolic left ventricular pressure, heart rate and coronary flow were assessed continuously as described (Dhein et al., 1993).

The delay between the end of the pacing stimulus and the first normal ventricular activation was assessed as PQ-time as a measure for the atrioventricular conduction time.

For evaluation of the mapping data the activation time points at each electrode were determined as $t(\mathrm{d}U/\mathrm{d}t_{\min})$ (Durrer and Van der Tweel, 1954; Dhein et al., 1993). Next, the repolarisation time points were determined as $t(\mathrm{d}U/\mathrm{d}t_{\max})$ during the T-wave as described (Millar et al., 1985; Dhein et al., 1993). From these data for each electrode the activation–recovery-interval was assessed as the epicardial action potential duration. The spatial distribution of the epicardial action potential durations was analysed for each area of the heart (i.e. front, left, right or back wall) calculating the standard deviation of the epicardial action potential durations at 64 electrodes and expressed as dispersion. From the activation time points an activation sequence was determined. We determined those electrodes which were activated before any of the neighbouring ones and defined them as 'breakthrough-points' which can be considered as the origins of epicardial activation (Arisi et al., 1983). These breakthrough-points were determined for heart beats under control conditions and for heart beats under treatment. Heart beats under various treatments (increasing concentrations of acetylsalicylic acid, or various phases of ischemia and reperfusion) were then compared to those under control conditions by calculating the percentage of breakthrough-points with identical location as compared to their location under control conditions (identical = deviating not more than 1 mm from their location under control conditions). That means, that two identical heart beats should reveal a breakthrough-point-similarity of 100%. It is, however known from previous studies, that identical heart beats do occur only rarely and that proarrhythmic stimuli reduce breakthrough-point-similarity (Dhein et al., 1988, 1989, 1990, 1993). In the above studies we defined a lower limit of 50% breakthrough-point-similarity beneath which it was only a matter of time until arrhythmia would occur.

In a similar way the spread of epicardial excitation was analysed. In order to allow a quantitative and comparative description of the activation process for each electrode an activation vector was calculated from the activation times and the locations of the surrounding electrodes which were activated after the central electrode (i.e. a maximum number of 8), as described by Müller et al. (1991). These vectors give direction and apparent velocity of local activation. The percentage of similar vectors between heart beats under increasing drug concentrations compared with those under control conditions was determined (vectors deviating not more than 5° from their original direction were considered to be similar). The critical value beneath which arrhythmia occurs (see above) for vectorfield-similarity is 10% as determined in previous studies (Dhein et al., 1988, 1989, 1990, 1993).

Taken together, the parameters breakthrough-point- and vectorfield-similarity characterise the geometry of the epicardial activation process, and represent the beat similarity of the cardiac impulse as compared to heart beats under control conditions. Thus, decreasing values for these similarities indicate progressive deviation from the initial (control) activation pattern.

In addition, the ST-segments of the 256 ECG's were analysed. We summed up all deviations from the isoelectrical level at the time point 50% of mean epicardial action potential duration and calculated the total ST-deviation of 256 leads in arbitrary units (au). An increase in that parameter points to an increase in efflux of positively charged ions during the action potential and is indicative for ischemic regions (Dhein et al., 1990a).

In further preliminary experiments we tested the influence of indomethacin (1 μmol/l) and of acetylsalicylic acid (0.5 μmol/l) on the cardiac cyclooxygenase activity in isolated rabbit hearts by infusion of 1 μmol/l arachi-

donic acid and measurement of 6-keto-$PGF_{2\alpha}$ in the effluent using a radioimmunoassay according to a standard method as described by Schrör and Seidel (1988). In accordance with the literature (Gryglewski, 1979) we observed a rise in 6-keto-$PGF_{2\alpha}$ to 1000–2500 pg/ml within 5 min and found an almost complete inhibition of 6-keto-$PGF_{2\alpha}$ formation under the influence of indomethacin but, as could be expected for this low concentration of acetylsalicylic acid, no inhibition by 0.5 μmol/l acetylsalicylic acid.

2.2. Histo-autoradiography

After perfusion of the hearts with 25 μCi ^{14}C-acetylsalicylic acid these hearts were perfused with 5% formalin (pH = 7, phosphate buffered) for fixation; the position of the electrodes was marked by injection of ink. After a 7 days postfixation period in 5% formalin, the marked ventricular areas under the electrode grids were excised, embedded in hydroxyethylmethacrylate (Technovit 7100; Heraeus-Kulzer, Wehrheim, FRG), cut into 2 μm thick sections, fixed to slides and dipped in NTB2-emulsion (emulsion: H_2O: 2:1 at 42°C; Kodak). After 100 days of exposure at −20°C the slides were developed and photographs were taken using a Zeiss Axiolab with Achroplan-objectives and a Nikon F3 camera (at magnification: 400×). These pictures were evaluated using a digital image analysis system (Data translation card and JAVA-software, Jandel Scientific, Erkrath, FRG). We counted the silver grains indicating radioactivity outside the section for background and in the right and left ventricle. 20 right and 20 left ventricular sections were evaluated in this way.

2.3. Statistics

All values are given as means ± S.E.M. of at least $n = 6$ experiments in each series. Significance was analysed using Wilcoxon rank test for paired observations, Mann-*U*-test for unpaired observations and analysis of variance for comparison of multiple groups, all on a level of $P < 0.05\%$.

2.4. Chemicals

All chemicals used in this study were of analytical grade. All chemicals were purchased from Sigma (St. Louis, USA), except heparin which was from Serva (Heidelberg, FRG). Acetylsalicylic acid was obtained as free acids. The aqueous solutions were freshly prepared before the experiments. 5 mg indomethacin were diluted (daily before the experiment) in 0.81 ml 0.5 mol/l Na_2HPO_4-solution, 0.19 ml 0.5 mol/l NaH_2PO_4-solution and 4.0 ml distilled water.

3. Results

The initial control values after 60 min equilibration under standard conditions are given in Table 1 for all experimental series. Under non-ischemic conditions application of acetylsalicylic acid resulted in a slight but significant increase in the epicardial action potential duration as shown in Table 2. We did not observe an increase in dispersion which remained in the range of 7–10 ms as calculated for all 256 electrodes (Table 2). Acetylsalicylic acid led to a concentration dependent moderate alteration of the activation patterns as described by a decrease in breakthrough-point-similarity (by 6% at 0.5 μmol/l; see Table 2) and in vectorfield-similarity (by 19% at 0.5 μmol/l; Table 2). The other parameters were not changed significantly by acetylsalicylic acid as compared to controls (see Table 2).

In all hearts submitted to regional ischemia the coronary flow was reduced by about 25% and recovered after release of the ligature as depicted in Fig. 1. There is no complete recovery of coronary flow to the preischemic control value for the whole reperfusion period, since the coronary flow is progressively decreasing in non-ischemic perfused hearts as can be seen from the time control series (Table 2). However, although significantly lower than the preischemic value the coronary flow during reperfusion was not significantly different from the respective time control values. It should be stated that in hearts receiving indomethacin we did not observe vasoconstriction (Fig. 1, column 'con' versus column 'drug'), which is known to occur sometimes after administration of high concentrations of this drug. In all hearts systolic left ventricular pressure was reduced by ischemia to the same degree, i.e. to 52–60% of the pre-ischemic control value (values after 20 min of ischemia: untreated control: 54 ± 6%, acetylsalicylic acid: 54 ± 4%, indomethacin: 53 ± 2%). The area in which ST-elevation was observed did not differ signifi-

Table 1
Control values for the series including regional ischemia/reperfusion with or without pre-treatment with 0.5 μmol/l acetylsalicylic acid (ASA) or 1.0 μmol/l indomethacin. All values are given as means ± S.E.M. of $n = 7$ experiments and $n = 6$ (indomethacin) (abbreviations: LVP: left ventricular pressure; CF: coronary flow; ST: elevation of ST-segment; BTP: similarity of breakthroughpoints; VEC: similarity of the vectorfields, PQ: atrioventricular conduction time; EAPD: epicardial action potential duration; Disp: dispersion = standard deviation of EAPD)

	Control	ASA	Indomethacin
LVP (mm Hg)	82 ± 5	89 ± 5	73 ± 9
CF (ml/min)	26 ± 3	31 ± 3	38 ± 1
ST (a.u.)	42 ± 16	40 ± 15	31 ± 9
BTP (%)	79 ± 6.8	69 ± 8	69.1 ± 7.9
VEC (%)	25.3 ± 3.4	25.0 ± 3.7	25.2 ± 4.7
PQ (ms)	63 ± 2	59 ± 1	62 ± 3
EAPD (ms)	128 ± 9	129 ± 4	110 ± 7
Disp (ms)	6.1 ± 1.8	5.6 ± 1.2	8.6 ± 2.1

Table 2
Effects of cumulative concentrations of acetylsalicylic acid (ASA) on the functional and electrophysiological parameters under observation as compared to the time control series. All values are given as means ± S.E.M. of $n = 7$ experiments

		Control (min) (ASA (μmol/l))				
		60	75	90	105	120
		0.0	0.05	0.1	0.5	1.0
LVP (mm Hg)	control	82 ± 4	75 ± 4	70 ± 4	64 ± 4	60 ± 5
	ASA	86 ± 2	75 ± 2	68 ± 2	64 ± 2	60 ± 3
CF (ml/min)	control	29 ± 2	28 ± 2	26 ± 2	25 ± 2	24 ± 2
	ASA	28 ± 2	28 ± 2	25 ± 2	24 ± 2	23 ± 1
ST (a.u.)	control	56 ± 11	50 ± 12	46 ± 9	37 ± 10	29 ± 6
	ASA	44 ± 12	50 ± 11	43 ± 11	39 ± 9	37 ± 8
BTP (%)	control	73 ± 3.1	77 ± 3.1	78 ± 3.2	77 ± 3.5	78 ± 3.0
	ASA	74 ± 2.5	73 ± 2.9	70 ± 3.4 *	68 ± 3.3 *	65 ± 3.7 *
VEC (%)	control	35.0 ± 2.6	31.6 ± 2.7	25.4 ± 2.5	24.4 ± 2.4	20.9 ± 2.2
	ASA	38.4 ± 2.5	23.4 ± 1.5 *	23.0 ± 2.2 *	19.5 ± 1.7 *	17.4 ± 1.5 *
PQ (ms)	control	63 ± 2	63 ± 2	62 ± 1	63 ± 2	63 ± 2
	ASA	64 ± 2	65 ± 2	65 ± 2	65 ± 2	64 ± 2
EAPD (ms)	control	135 ± 3	132 ± 3	136 ± 2	141 ± 2	138 ± 2
	ASA	119 ± 2	118 ± 2	124 ± 3 *	125 ± 2 *	129 ± 2 *
Disp (ms)	control	10.2 ± 1.0	8.6 ± 0.9	9.0 ± 1.1	8.8 ± 0.9	9.2 ± 1.0
	ASA	9 ± 1.1	8.1 ± 1.1	8.2 ± 0.9	8.6 ± 1.0	7.9 ± 0.8

Abbreviations: LVP: left ventricular pressure; CF: coronary flow; ST: elevation of ST-segment; BTP: similarity of breakthrough-points; VEC: similarity of the vectorfields, PQ: atrioventricular conduction time; EAPD: epicardial action potential duration; Disp: dispersion = standard deviation of EAPD).
* Significant differences ($p < 0.05$) between the changes in the time control series and the acetylsalicylic acid concentration–response curve.

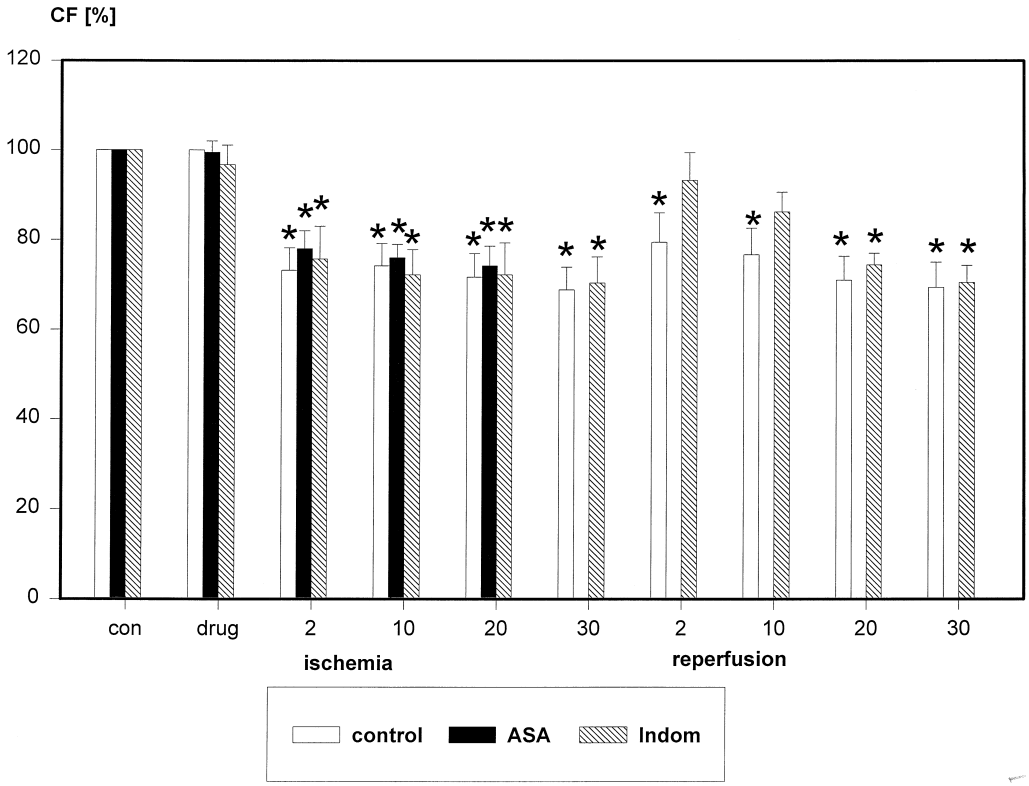

Fig. 1. Effects of 30 min regional ischemia and 30 min reperfusion with and without (= control) pre-treatment with 0.5 μmol/l acetylsalicylic acid (ASA) or with 1.0 μmol/l indomethacin (Indom.) on coronary flow (CF) in (%) of the preischemic control value after 60 minutes equilibration. All values are given as means ± S.E.M. of $n = 7$ experiments (control, ASA) and $n = 6$ (indomethacin). Significant changes as compared to control conditions after 60 min equilibration (= con) are indicated by an asterisk. After 20–30 min all hearts receiving acetylsalicylic acid developed sustained ventricular fibrillation, so that values for the experimental series with acetylsalicylic acid cannot be given for time points later than 20 min ischemia.

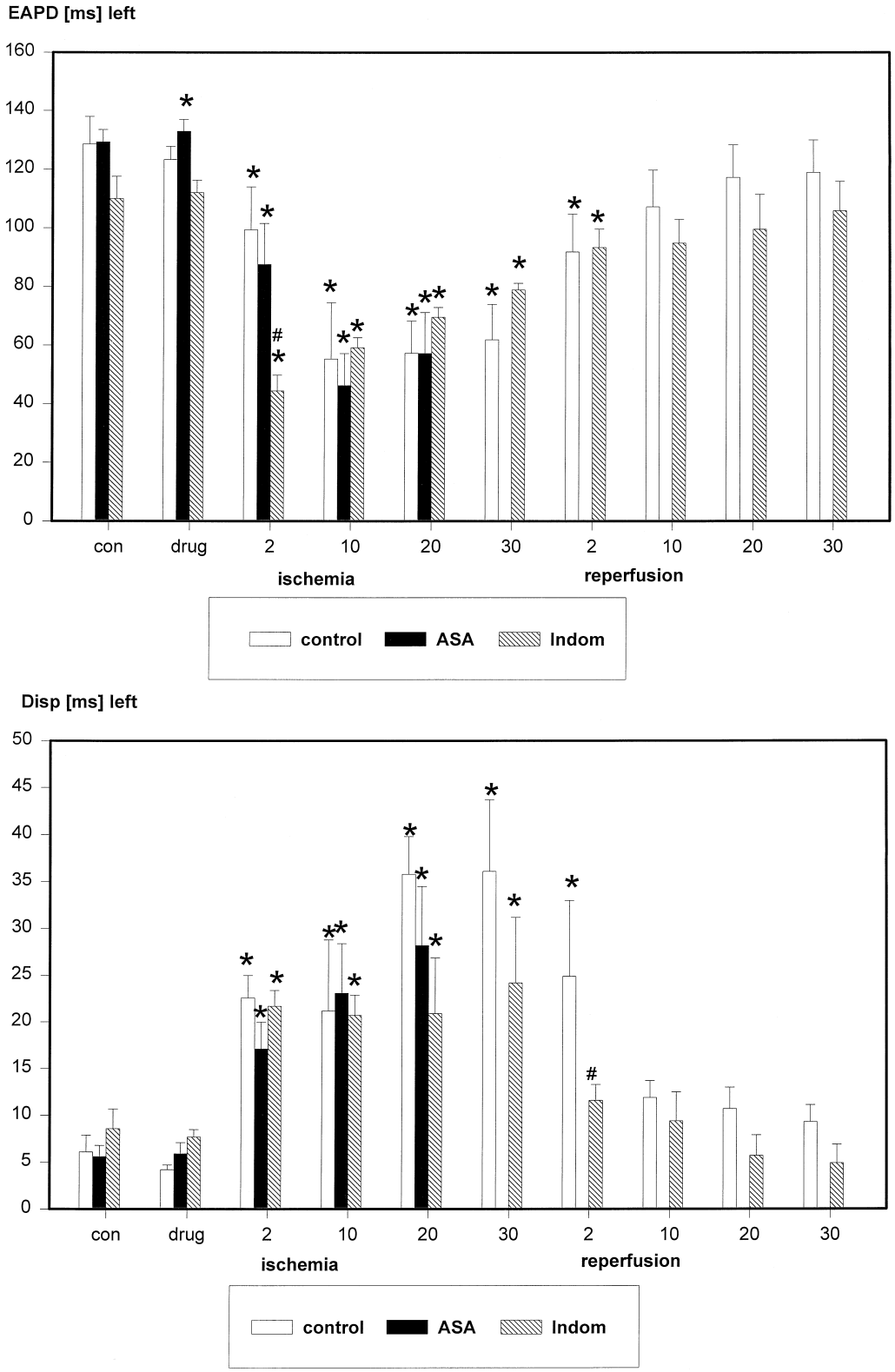

Fig. 2. Effects of 30 min regional ischemia and 30 min reperfusion with and without (= control) pre-treatment with 0.5 μmol/l acetylsalicylic acid (ASA) or with 1.0 μmol/l indomethacin (Indom.) on the left ventricular epicardial action potential duration (EAPD, upper panel) and on its dispersion (Disp, lower panel). All values are given as means $\pm$ S.E.M. of $n = 7$ experiments (control, ASA) and $n = 6$ (indomethacin). Significant changes as compared to control conditions after 60 min equilibration (= con) are indicated by an asterisk. Differences between the control series without pre-treatment and the series with treatment are marked by a '#'. After 20–30 min all hearts receiving acetylsalicylic acid developed sustained ventricular fibrillation, so that values for this experimental series cannot be given for time points later than 20 min ischemia.

cantly between these series, i.e. in all series an ischemic area of 30 ± 5 mm^2 was observed (values at 20 min ischemia). However, in all hearts (7/7) receiving acetylsalicylic acid sustained ventricular fibrillation occurred after 20–30 min ischemia. In contrast, the other hearts did not develop ventricular fibrillation in the course of the ischemic period.

During reperfusion only some of the hearts (2/7) of the control series and 2/6 of the hearts of the indomethacin series developed sustained ventricular fibrillation. Types of arrhythmia occurring during the entire experimental protocol in the three groups are summarised in Table 3.

In control hearts submitted to regional ischemia the epicardial action potential duration was markedly reduced at the left ventricular wall. This was similar in hearts receiving indomethacin or acetylsalicylic acid (Fig. 2, upper panel), except an enhanced shortening of the epicardial action potential duration at 2 min ischemia under the influence of indomethacin. After release of the ligation the epicardial action potential duration recovered within 10 minutes to values not significantly different from the initial values. As expected, the dispersion of the epicardial action potential duration within the left ventricular wall was increased during ischemia in all series (Fig. 2, lower panel) and recovered within 10 min of reperfusion.

Regional ischemia resulted in a marked alteration of the epicardial activation patterns as can be seen from the decrease in the percentage of those vectors which exhibit the same direction as before ischemia. In early ischemia (i.e. 2–10 min) this was significantly enhanced in hearts receiving either indomethacin or acetylsalicylic acid (Fig. 3). We found only incomplete recovery of the vectorfield-similarity during 30 min reperfusion under control conditions. This was not influenced by indomethacin.

In addition, regional ischemia led to a marked alteration of the ST-segments in the unipolar epicardial leads and thereby to an increase in ST. This was not influenced significantly by indomethacin but was significantly ($P < 0.05$) more pronounced (3-fold at 20 min ischemia) in the presence of acetylsalicylic acid (Fig. 4). We found an almost complete recovery of ST, of the epicardial action potential durations and of their dispersion after 20 min of reperfusion under control conditions. This was not altered by indomethacin. Since all hearts receiving acetylsalicylic

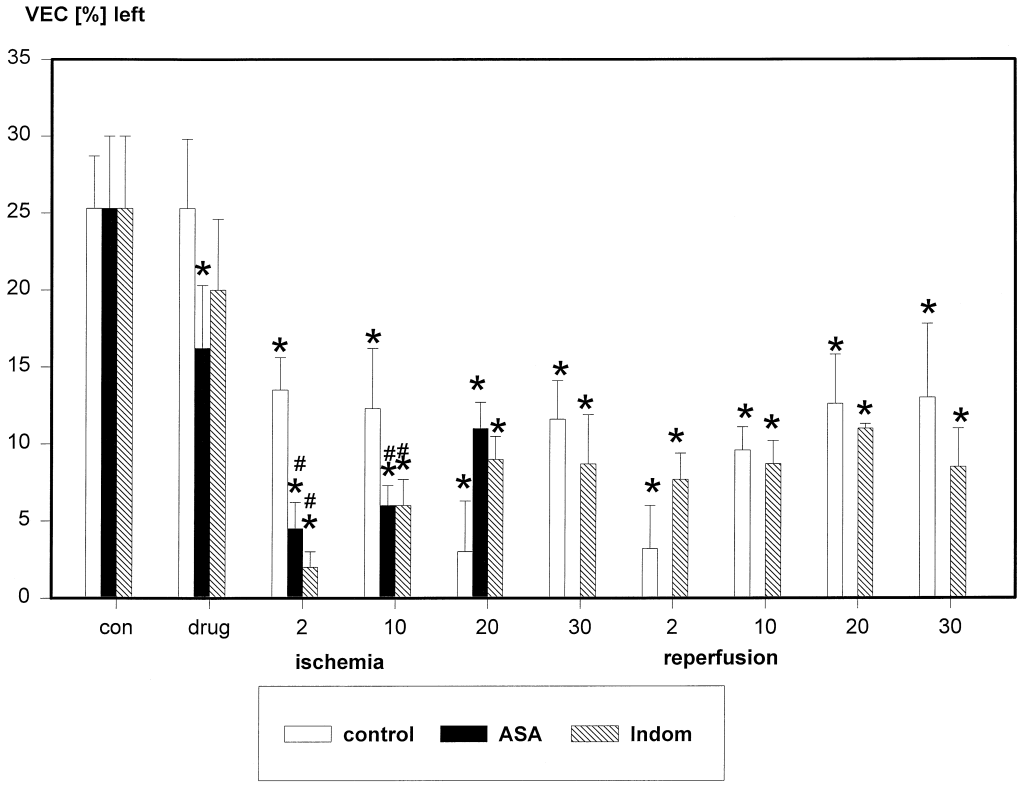

Fig. 3. Effects of 30 min regional ischemia and 30 min reperfusion with and without (= control) pre-treatment with 0.5 μmol/l acetylsalicylic acid (ASA) or with 1.0 μmol/l indomethacin (Indom.) on the percentage of activation vectors at the left ventricular wall which do not deviate more than 5° from the direction of the vector before induction of ischemia (= % VEC). All values are given as means ± S.E.M. of $n = 7$ experiments (control, ASA) and $n = 6$ (indomethacin). Significant changes as compared to control conditions after 60 min equilibration (= con) are indicated by an asterisk. Differences between the control series without pre-treatment and the series with treatment are marked by a '#'. After 20–30 min all hearts receiving acetylsalicylic acid developed sustained ventricular fibrillation, so that values for this experimental series cannot be given for time points later than 20 min ischemia.

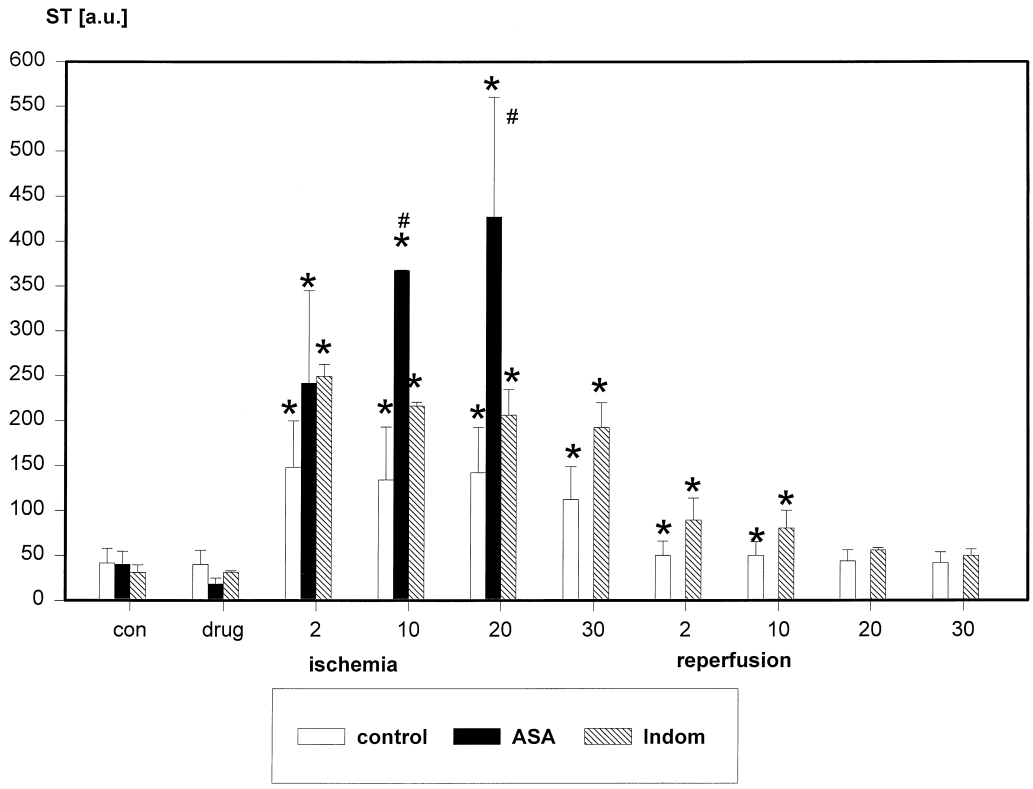

Fig. 4. Effects of 30 min regional ischemia and 30 min reperfusion with and without (= control) pre-treatment with 0.5 μmol/l acetylsalicylic acid (ASA) or with 1.0 μmol/l indomethacin (Indom.) on the ST-segment (ST) in arbitrary units [au] (for details see Section 2). All values are given as means ± S.E.M. of $n = 7$ experiments (control, ASA) and $n = 6$ (indomethacin). Significant changes as compared to control conditions after 60 min equilibration (= con) are indicated by an asterisk. Differences between the control series without pre-treatment and the series with treatment are marked by a '#'. After 20–30 min all hearts receiving acetylsalicylic acid developed sustained ventricular fibrillation, so that values for this experimental series cannot be given for time points later than 20 min ischemia.

acid developed sustained ventricular fibrillation during ischemia, there are no reperfusion data available for these hearts.

In all hearts perfused with ^{14}C-acetylsalicylic acid a significant accumulation of ^{14}C-acetylsalicylic acid in the ischemic region was observed (Fig. 5).

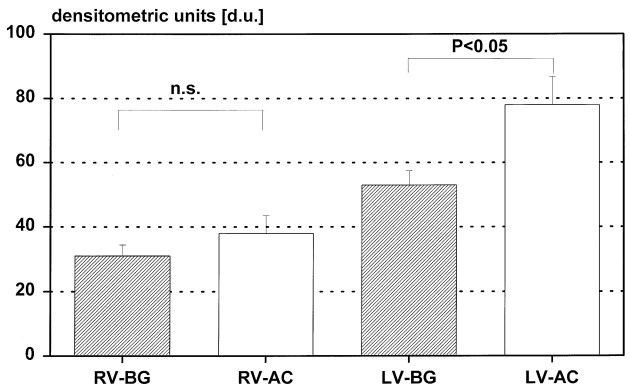

Fig. 5. Accumulation of ^{14}C-acetylsalicylic acid in the ischemic left ventricle after pre-treatment with 0.5 μM ^{14}C-acetylsalicylic acid (LV-AC) using histoautoradiography (for details see Section 2). For reference the background counts (LV-BG) are given and the values for the non-ischemic right ventricle (RV-AC; RV-BG). Section of 10 μm thickness were used. Values are given for $n = 6$ experiments as means ± S.E.M. in densitometric units (d.u.). Significance is indicated in the figure.

Table 3
Incidence of arrhythmia in the course of a 30 min LAD occlusion (ischemia) and 30 min reperfusion in hearts receiving no treatment (control, $n = 7$), 0.5 μmol/l acetylsalicylic acid (ASA, $n = 7$) or 1 μmol/l indomethacin (Indom., $n = 6$). The number of hearts in each experimental phase is given, which exhibit arrhythmia. Arrhythmia (ventricular extrasystoles) were classified according to the classification of Lown and Wolf (VF = ventricular fibrillation, which was in all cases sustained)

Series	Type	Con	Ischemia (min)				Reperfusion (min)			
			2	10	20	30	2	10	20	30
Control	I									
	II				1		4	4	3	
	III				1	2	2	2	1	
	IVa						1		1	
	IVb									
	VF						1	1	2	2
ASA	I									
	II		2							
	III		1	1						
	IVa			4	2					
	IVb				1					
	VF				4	7	7	7	7	7
Indom.	I									
	II									
	III					4	2	2		2
	IVa						2			
	IVb									
	VF								2	2

4. Discussion

The most striking finding in this study was that acetylsalicylic acid induced sustained ventricular fibrillation and led to enhanced ST-deviation during regional ischemia whereas treatment with indomethacin did not cause these changes. In addition, we found an accumulation of acetylsalicylic acid in the ischemic region. Acetylsalicylic acid slightly prolonged the epicardial action potential duration under control conditions.

Since this high incidence of sustained ventricular fibrillation during late ischemia in acetylsalicylic acid treated hearts was accompanied by an enhanced increase in ST-deviation and since this reflects an enhanced efflux of positive charges (probably K^+; Coronel et al., 1988, 1992; Dhein et al., 1990a, 1995), it can be imagined that this increase in ST-deviation may indicate a higher damage or an enhanced K^+-efflux which may in turn cause ventricular fibrillation. This effect seems to be independent from cyclooxygenase blockade since it could not be observed with indomethacin (see below). On the other hand, this enhancement of ischemia-induced ST-elevation by acetylsalicylic acid was not due to hemodynamic effects of the drug since there were no differences in coronary flow reduction between the experimental series (i.e. in all series coronary flow was reduced during occlusion by about 25%). In addition, there was no difference between the series with regard to the reduction in left ventricular pressure or to the ischemic area, i.e. the areas with ST-elevation during ischemia did not differ between the series. Thus, direct vascular actions do not seem to be the basis of the observed effects, rather than an enhanced damage, i.e. not the area of ischemia (or the area of ST-elevation) itself, but possibly the degree of damage (as indicated by the degree of ST-elevation) within that area seemed to be enhanced by acetylsalicylic acid.

It is known from previous studies (Tan et al., 1993) that intercellular communication is disturbed during late ischemia, probably because of increased gap junctional uncoupling. The possible causes for this include the lowering of intracellular pH, the rise in intracellular pCO_2, the loss of potassium and ATP, and the depolarisation as well as the intracellular calcium overload (Page, 1992; Steendijk et al., 1993). The facts that sustained ventricular fibrillation under acetylsalicylic acid occurred during late phase ischemia, that acetylsalicylic acid accumulates in the ischemic zone, and that ST-elevation was enhanced under acetylsalicylic acid indicating an enhanced efflux of positive charges, suggest the possibility that the ischemia-induced alteration of cellular communication may be enhanced in the presence of acetylsalicylic acid.

Comparing the effects of acetylsalicylic acid and indomethacin, it should be kept in mind that IC_{50} for acetylsalicylic acid actions on human platelets is in the order of 1.7 μmol/l, but in other tissues is at least one or two magnitudes higher, i.e. > 15 μmol/l (Gryglewski, 1979). Thus, acetylsalicylic acid in concentrations lower than 1 μmol/l probably does not inhibit myocardial cyclooxygenases, as indicated by our control experiments (see Section 2), whereas indomethacin in concentration of 1.0 μmol/l is known to inhibit cyclooxygenase (Lee, 1974) as could be verified by our control experiments (see methods section) and according to Gryglewski (1979) does not affect other enzyme systems. Thus, it can be imagined that the acetylsalicylic acid-induced arrhythmogeneity may not be due to cyclooxygenase inhibition, since indomethacin did not cause these arrhythmia.

The accumulation of acetylsalicylic acid in the ischemic region may be due to the acidification of the ischemic region thereby facilitating the penetration of acetylsalicylic acid which has a pK_a of about 3.5 (Merck Index, 1989). On the other hand this accumulation of an organic acid may lower intracellular pH further, alter the intercellular communication as discussed above and the intracellular metabolism or may enhance the cellular damage. Since indomethacin has a higher pK_a of about 4.5 (Merck Index, 1989) it should not accumulate in the same way.

Under non-ischemic conditions we observed a slight prolongation of the epicardial action potential duration by acetylsalicylic acid and a very weak prolongation by indomethacin ($5 \pm 1\%$ with 0.5 μmol/l acetylsalicylic acid versus $2.3 \pm 1.7\%$ with 1.0 μmol/l indomethacin). Since PGI_2 has been shown to shorten cardiac action potential duration (Müller et al., 1995), the prolongation of the epicardial action potential duration may be in parts due to a lack of PGI_2 especially in indomethacin treated hearts, whereas because of the lack of effect of this low concentration of acetylsalicylic acid on the cardiac cyclooxygenase in acetylsalicylic acid-treated hearts other effects on the repolarisation may be assumed as suggested previously (Kwiatkowska-Patzer and Herbaczynska-Cedro, 1981).

On the other hand, under the influence of acetylsalicylic acid or indomethacin there was no prolongation of the epicardial action potential durations observable during ischemia as compared to untreated ischemic hearts. Thus, the prolongation of the epicardial action potential durations under non-ischemic conditions obviously is counteracted by some other effect of acetylsalicylic acid during ischemia the molecular mechanism of which remains unknown yet.

In the reperfusion phase of the experiments, we did not observe an influence of indomethacin on recovery.

In summary, acetylsalicylic acid exhibited proarrhythmic effects during late ischemia whereas this was not observed with indomethacin. Since in addition acetylsalicylic acid, but not indomethacin, enhanced the ischemia-induced ST-elevation, we propose, that the proarrhythmic action of acetylsalicylic acid is independent from cyclooxygenase inhibition (as in addition supported by our measurements of cyclooxygenase activity). It may be due to some other effects, for example enhanced uncoupling by

accumulation of an organic acid, which have to be elucidated in further studies.

We conclude that acetylsalicylic acid exerts cardiac electrophysiological effects resulting in action potential prolongation and alteration of the epicardial activation pattern in non-ischemic hearts. It is further concluded that acetylsalicylic acid can accumulate in the ischemic region and induce ventricular fibrillation during regional ischemia. Thus, acetylsalicylic acid, in spite of its well known beneficial antiaggregatory effects exerts electrophysiological effects which are proarrhythmic at least in this rabbit model. These effects are at least partially independent from cyclooxygenase blockade, since they were not observed with indomethacin. In most clinical situations these effects of acetylsalicylic acid probably are dominated by its undoubtedly beneficial antiaggregatory effects. However, it might be that other antiaggregatory agents may have lower proarrhythmic side effects. This problem may especially play a role if acetylsalicylic acid is injected intravenously in acute situations like unstable angina pectoris or acute myocardial infarction. Our findings may help to explain the increased incidence of sudden death under aspirin therapy reported by others (Colwell et al., 1986; Chimowitz et al., 1995).

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