Virchows Archiv A Pathol Anat (1990) 417:463–471

Virchows Archiv A
Pathological Anatomy and Histopathology

Occurrence and prevention of contraction bands in Purkinje fibres, transitional cells and working myocardium during global ischaemia

Ph.A. Schnabel[1], A. Schmiedl[1], B. Ramsauer[1], U. Bartels[1], M.M. Gebhard[1], J. Richter[2], and H.J. Bretschneider[1]

[1] Abteilung Vegetative Physiologie und Pathophysiologie, Zentrum Physiologie und Pathophysiologie,
[2] Abteilung Elektronenmikroskopie, Zentrum Anatomie, Universität Göttingen, Göttingen, Federal Republic of Germany

Received April 24, 1990 / Received after revision July 31, 1990 / Accepted July 31, 1990

Summary. Contraction bands usually occur in the intramural working myocardium following post-ischaemic reperfusion. In the subendocardium, however, they are found during ischaemia. Thus, we ascertained the contraction states of Purkinje fibres, transitional cells, subendocardial and intramural parts of the working myocardium during 30 min global ischaemia at 25° C. The effects with and without myocardial protection were compared. At the onset of pure ischaemia contraction bands are completely lacking in all cell types. During pure ischaemia contraction bands are found in all subendocardial cell types but not in the intramural working myocardium. A peak of pathological contraction states is found in the intramural working myocardium at the onset (0 min), in the subendocardial working myocardium at 10 min, in the transitional cells and Purkinje fibres at 30 min of pure ischaemia. **H**istidine-, **t**ryptophan-, **k**etoglutarate-enriched (HTK) cardioplegia prevents contraction bands completely at the onset of ischaemia and prevents both contraction bands and pathological contraction states during ischaemia almost completely. Striking differences in the physiological contraction states are seen only in the working myocardium: HTK cardioplegia brings about dominance of relaxation during ischaemia. These findings may be due mainly to the effects of global ischaemia on the one hand and to catecholamines, calcium and oxygen on the other.

Key words: Purkinje fibres – Transitional cells – Working myocardium – Global ischaemia – Ultrastructure – Contraction state

Introduction

Contraction bands are a typical sign of myocardial damage occurring during post-ischaemic reperfusion, but even during prolonged periods of ischaemia contractures may be observed (Ganote 1983; Karch and Billingham 1985; Todd et al. 1985; Armiger and Smeeton 1986; Vander Heide et al. 1986; Allen and Orchard 1987; Anderson et al. 1987; Elz and Nayler 1988). Generally, contraction bands are reported only in the working myocardium; however, Armiger et al. (1979) discovered them in the trabecula septomarginalis. A transmural gradient is found for ischaemic alterations and post-ischaemic myocardial dysfunction, with the most severe alterations localized subendocardially (Lowe et al. 1983; Anderson et al. 1987; Bolli et al. 1989). Similarly, contraction bands following sudden cardiac death or due to catecholamines are situated in the subendocardium preferentially (Arnold et al. 1985; Todd et al. 1985; Armiger and Smeeton 1986).

Contraction bands can be due to disturbances of the microcirculation, or may cause them by compressing the capillary bed and increasing coronary resistance to the point of "no reflow" (Appelbaum et al. 1983; Bretschneider et al. 1983; Anderson et al. 1987; Schnabel et al. 1987). In the subendocardium, diffusion of oxygen via the endocardium may partly compensate for low-flow or no-flow situations occurring during regional or global ischaemia, depending on the myocardial oxygen consumption (Bretschneider 1961; Hearse 1988; Gebhard and Bretschneider 1989). Nevertheless, post-ischaemic rhythm disturbances can originate from subendocardial Purkinje fibres (Friedman et al. 1975; Gettes 1986). In global ischaemia and following post-ischaemic recovery after different forms of cardiac arrest, contraction bands and striking alterations of the contraction state were recently described in these subendocardial Purkinje fibres (Schnabel et al. 1988a, b; Ramsauer et al. 1989). In contrast, during ischaemia prolonged to the point of reaching irreversibility contraction bands were not found in the intramural working myocardium (Schmiedl et al. 1990a, c).

Thus, in this study we investigated the contraction state of the sarcomeres in the subendocardium qualitatively and quantitatively. The alterations in Purkinje

Offprint requests to: Ph.A. Schnabel, Zentrum Physiologie und Pathophysiologie, Humboldtallee 23, W-3400 Göttingen, Federal Republic of Germany

fibres, transitional cells and subendocardial parts of the working myocardium were compared with the intramural working myocardium. The proportion of physiological and pathological contraction states was ascertained during 30 min of ischaemia (25° C) with myocardial protection (HTK cardioplegia) and without (pure ischaemia).

Materials and methods

Experimental procedures, anaesthesia, fixation and further processing of the samples have been described recently (Schnabel et al. 1987, 1990a, b; Schmiedl et al. 1990a). Two groups of at least five canine hearts were arrested in situ by inflow occlusion, aortic cross-clamping and for pure ischaemia by topical cooling with ice-cold Tutofusin solution, or (HTK cardioplegia) by 11 min coronary perfusion with Bretschneider's cardioplegic solution HTK at 8° C as performed clinically (Preusse et al. 1987).

The composition of Tutofusin in mmol/l amounts to 140 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$ (Pfrimmer, Erlangen, FRG); for HTK it is 15 NaCl, 9 KCl, 4 $MgCl_2$, 180 *l*histidine, 18 histidine-HCl, 2 *l*tryptophan, 1 K-α-*k*etoglutarate, 30 mannitol (Dr. F. Koehler Chemie GmbH, Alsbach, FRG).

Samples were taken in situ at the onset of ischaemia and following incubation of the left ventricle in the solution employed for cardiac arrest after 10 and 30 min of ischaemia at 25° C. Samples from the subendocardial third of the free ventricular wall were immediately dissected in the fixative with razor blades so that the relationship between subendocardial Purkinje fibres, transitional cells and working myocardium remained intact as far as possible during fixation (Richter et al. 1986; Schnabel et al. 1988a, 1990b).

Three random sections of three random tissue blocks were evaluated per heart and time point. All qualitative and quantitative measurements were carried out on-line with an EM 10 (Zeiss, Oberkochen, FRG) equipped with a TV camera. Following systematic random sampling (Weibel 1979), 30 fields per section were analysed for the contraction state of the intramural working myocardium (in the free left ventricular wall at a distance of 400 ± 100 µm from the endocardium; Schmiedl et al. 1990a). In the subendocardial working myocardium (the first two layers of working myocardium adjacent to the Purkinje fibres) and in the subendocardial Purkinje fibres, the test system was usually applied 20 times per section, because of the limited number of differing cells. In the case of the transitional cells, application was sometimes less than this depending on the quantity of these cells present.

The analysis of the contraction state was carried out at a final magnification of ×14000 using an area of 230 µm² per test field. Assessment of the contraction state of the sarcomeres of each test field was based on the following classification into five groups:

1. Relaxation = I-bands present in all sarcomeres.
2. Contraction = I-bands partially or completely absent, no A-band compression.
3. Overcontraction = A-band compression, no actin-myosin clumping.
4. Hypercontraction = A-band compression, actin-myosin clumping present, no ruptures of the contractile apparatus.
5. Contraction band = actin-myosin clumping, rupture of the contractile system, sometimes even rupture of the sarcolemma, nuclear membrane or outer mitochondrial membrane.

Groups 1 and 2 are considered together as physiological contraction states, groups 3, 4 and 5 as pathological states.

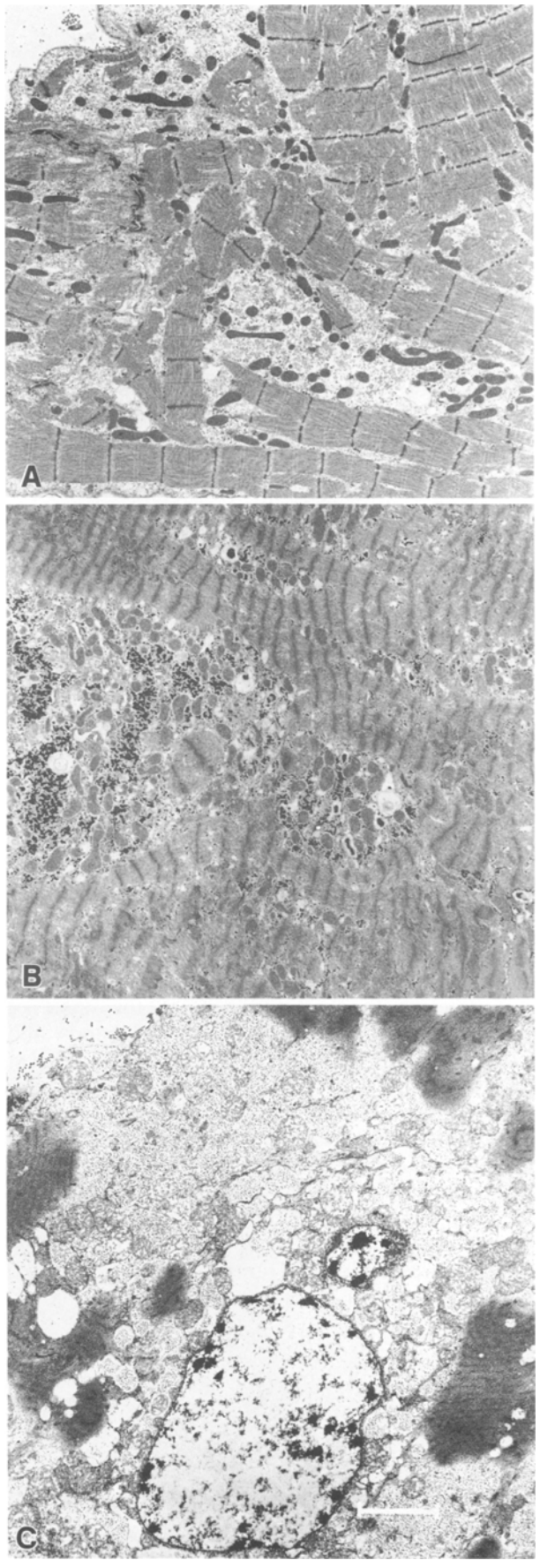

Fig. 1 A–C. Subendocardial Purkinje fibres of the left ventricle. **A** HTK cardioplegia, 30 min ischaemia (25° C); **B** pure ischaemia, onset of ischaemia; **C** pure ischaemia, 30 min ischaemia (25° C). ×5600; *scale bar* = 2 µm

All results are given as mean values ±SEM unless indicated otherwise. Significant differences have been noted for P values of 0.05 or less using the Wilcoxon-Mann-Whitney (U) test, for unpaired samples or the Student's t-test for paired samples.

Results

The ultrastructure and contraction state of the intramural working myocardium have been described earlier (Schmiedl et al. 1990a; Schnabel et al. 1990a). In this study the contraction states of the different subendocardial cell types are demonstrated.

In subendocardial Purkinje fibres (Fig. 1) the myofibrils are orientated in a three-dimensional network and are not always cut longitudinally. Following HTK cardioplegia and 30 min of ischaemia at 25° C (Fig. 1A) the sarcomeres are either relaxed, showing narrow I-bands or are contracted. Some of the Z-bands show irregular thickening typical of normal Purkinje fibres. At the onset of pure ischaemia, immediately after aortic cross-clamping (Fig. 1B) some of the Purkinje fibres show overcontractions. A-band compression and relatively homogeneous Z-band thickening are apparent. After 30 min of pure ischaemia (Fig. 1C) contraction bands lead to striking deterioration of the Purkinje fibre fine structure. The clumped actin-myosin filaments are aggregated in some places and separated by large areas free from myofibrils, indicating the rupture of the contractile system. Moreover, swelling and further alterations of the mitochondria, as well as clumping of nuclear chromatin are severely pronounced.

In transitional cells (Fig. 2) the myofibrils are more regularly aligned. HTK cardioplegia and a subsequent 30 min of ischaemia at 25° C (Fig. 2A) bring about clearly relaxed sarcomeres. In the centre of the cell there are some Z-band irregularities, seldom found in transitional cells. Following 30 min of pure ischaemia (Fig. 2B), many transitional cells show relaxation, whereas others (Fig. 2C) reveal pathological contraction states, in this case mainly overcontractions.

The different cell types in the subendocardium (Fig. 3) which are subjected to largely similar ambient conditions often show different contraction states. The stress of 30 min pure ischaemia at 25° C (Fig. 3A) brings about a contraction in the Purkinje fibres, and a contraction or an overcontraction in the transitional cells. After 10 min of pure ischaemia (Fig. 3B) the Purkinje fibre and the transitional cell are contracted, whereas the working myocardium is relaxed. There are also differences in the preservation of the mitochondria which are clearly less altered in the working myocardium. For the same period of pure ischaemia (Fig. 3C) another layer of transitional cells reveals contraction in one cell and paradiscal overcontractions in the other. The adjacent working myocardium shows relaxed or contracted sarcomeres.

In the subendocardial working myocardium (Fig. 4) the myofibrils are regularly aligned and tightly packed. At the onset of pure ischaemia (Fig. 4A) a considerable part of the subendocardial working myocardium is relaxed. After 10 min of pure ischaemia at 25° C (Fig. 4B)

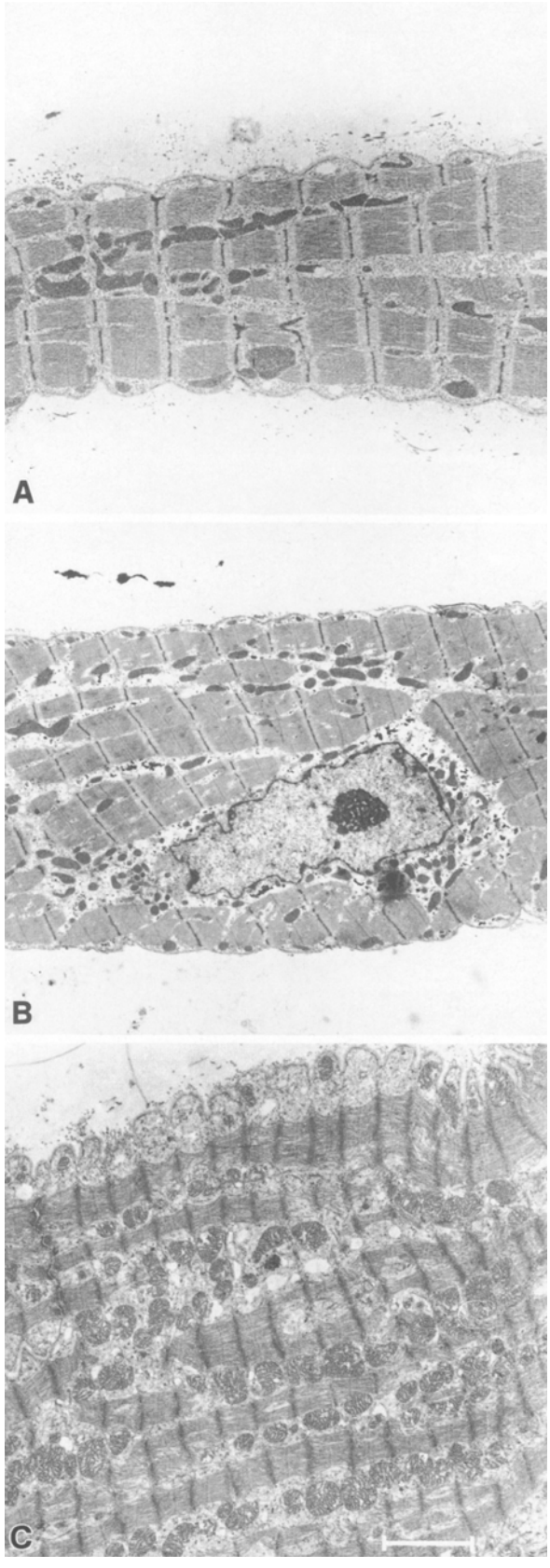

Fig. 2A–C. Subendocardial transitional cells of the left ventricle. **A** HTK cardioplegia, 30 min ischaemia (25° C); **B** pure ischaemia, 30 min ischaemia (25° C); **C** pure ischaemia, 30 min ischaemia (25° C). ×5600; *scale bar* = 2 μm

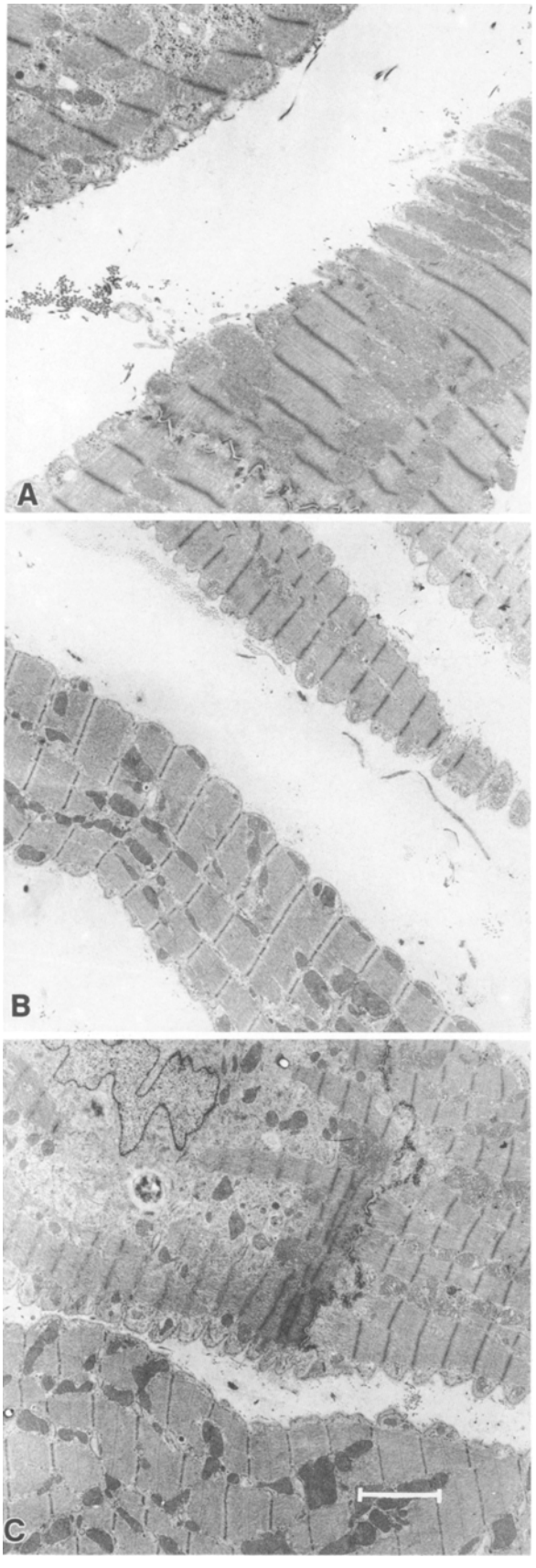

Fig. 3A–C. Different cell types in the subendocardium of the left ventricle. **A** Purkinje fibre (*upper left corner*) and transitional cell (*lower right corner*), pure ischaemia, 30 min ischaemia (25° C); **B** Purkinje fibre (*upper right corner*), transitional cell (*middle*) and working myocardium (*bottom*), pure ischaemia, 10 min ischaemia (25° C); **C** transitional cell (*top*) and working myocardium (*bottom*), pure ischaemia, 10 min ischaemia (25° C). ×5600; *scale bar* = 2 μm

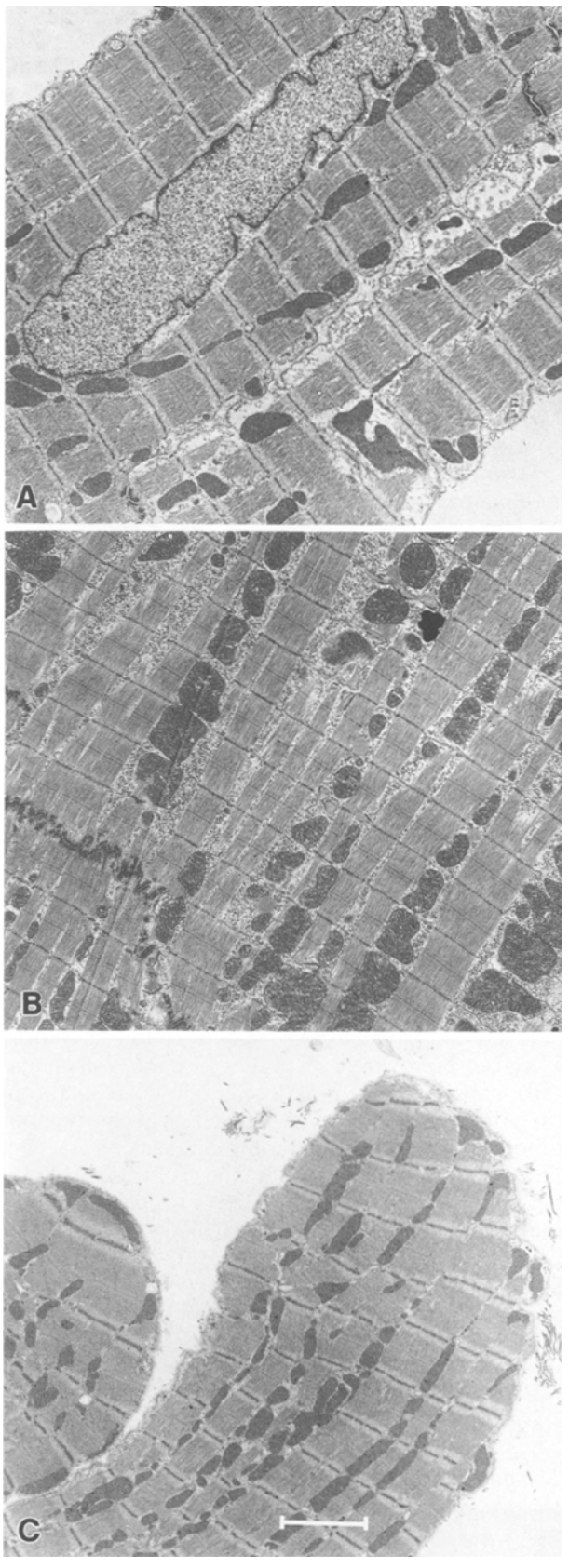

Fig. 4A–C. Subendocardial working myocardium of the left ventricle. **A** Pure ischaemia, onset of ischaemia; **B** pure ischaemia, 10 min ischaemia (25° C); **C** HTK cardioplegia, onset of ischaemia. ×5600; *scale bar* = 2 μm

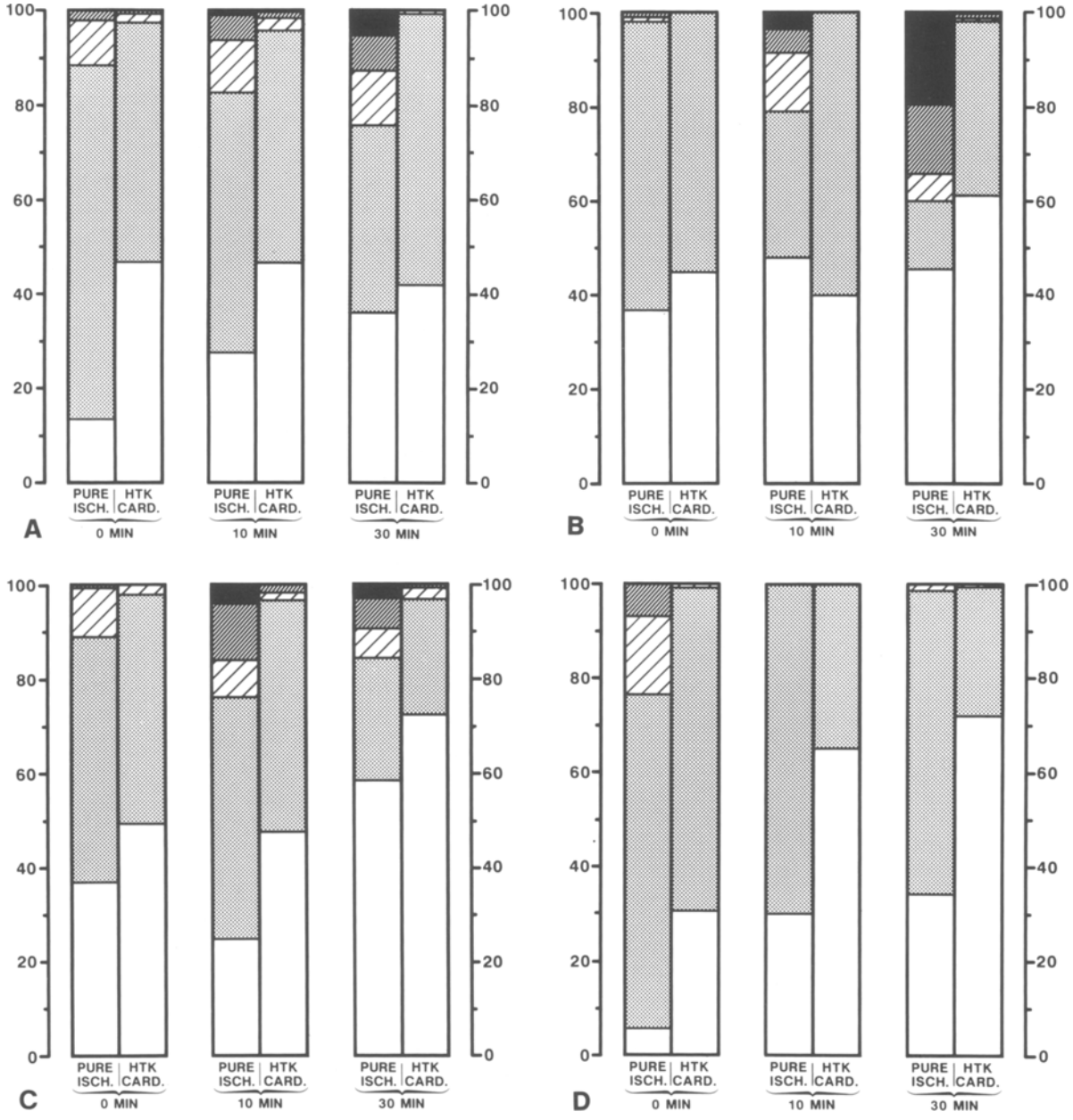

Fig. 5A–D. Contraction states [%] of the different cell types or layers of the left ventricular subendocardium during global ischaemia at 25° C: **A** subendocardial Purkinje fibres; **B** subendocardial transitional cells; **C** subendocardial working myocardium; □ Relaxation ▩ Contraction ▨ Overcontraction ▨ Hypercontraction ■ Contraction Band ($\bar{x}$, $n=5$). **D** intramural working myocardium; □ Relaxation ▩ Contraction ▨ Overcontraction ▨ Hypercontraction ($\bar{x}$, $n=6$). (Isch. = ischaemia, card. = cardioplegia)

many sarcomeres are contracted; some show very narrow I-bands. At the onset of ischaemia immediately after HTK cardioplegia (Fig. 4C) the subendocardial working myocardium is largely relaxed.

For Purkinje fibres (Fig. 5A, Table 1) at the onset of pure ischaemia quantitative examination shows the majority of the sarcomeres to be contracted, a much smaller proportion are relaxed, somewhat fewer are overcontracted, and only very few are hypercontracted. Ten minutes of pure ischaemia at 25° C leads to a slight increase in relaxed and in pathologically contracted sarcomeres. After 30 min of pure ischaemia, the number of relaxed and the number of pathologically contracted sarcomeres ($P<0.05$) have doubled, contracted sarcomeres are significantly ($P<0.01$) reduced.

Immediately following the end of HTK perfusion, at the onset of ischaemia, about equal numbers of the sarcomeres of Purkinje fibres are relaxed and contracted. Overcontractions and hypercontractions are very seldom seen. Thirty minutes of ischaemia (25° C) after HTK perfusion leads to very slight changes: relaxed sarcomeres decrease and contracted sarcomeres increase; overcontractions are found in less than 1%; hypercontractions and contraction bands are lacking entirely. Thus, compared to 30 min of pure ischaemia, HTK cardioplegia brings about a significantly higher percentage

Table 1. Contraction states of the sarcomeres during global ischaemia at 25° C

Time of ischaemia [min]	0		10		30	
	Pure ischaemia	HTK cardioplegia	Pure ischaemia	HTK cardioplegia	Pure ischaemia	HTK cardioplegia
Purkinje fibres ($n=5$)						
Relaxation	13 ±2	47 ±6	28±6	47 ±7	36±10	42 ±7
Contraction	75 ±8	51 ±5	55±5	49 ±6	39±7	58 ±7
Overcontraction	10 ±7	2 ±0.6	11±3	3 ±2	12±4	0.3±0.3
Hypercontraction	2 ±1	0.2±0.2	5±4	1 ±0.7	8±2	–
Contraction bands	–	–	1±0.5	0.3 ±0.3	5±4	–
Transitional cells ($n=5$)						
Relaxation	37 ±16	45 ±16	48±14	40 ±11	45±12	61 ±10
Contraction	61 ±15	55 ±16	31±10	60 ±11	15±6	37 ±10
Overcontraction	1 ±1	–	12±8	–	6±3	0.4±0.4
Hypercontraction	1 ±1	–	5±5	–	15±6	0.8±0.8
Contraction bands	–	–	4±4	–	19±8	0.4±0.4
Subendocardial working myocardium ($n=5$)						
Relaxation	37 ±8	50 ±8	25±7	48 ±7	58±12	72 ±6
Contraction	52 ±9	48 ±8	51±11	51 ±7	27±10	25 ±6
Overcontraction	11 ±7	2 ±1	8±2	0.3±0.3	6±2	2.5±2.5
Hypercontraction	0.3±0.3	–	12±6	0.5±0.5	6±4	0.5±0.5
Contraction bands	–	–	4±3	–	3±2	–
Intramural working myocardium ($n=6$)						
Relaxation	6 ±4	31 ±8	30±11	65 ±11	34±6	72 ±6
Contraction	71 ±7	68 ±8	70±11	35 ±11	65±7	28 ±6
Overcontraction	16 ±5	1 ±0.4	–	–	1±0.7	0.1±0.1
Hypercontraction	7 ±4	–	–	–	–	–
Contraction bands	–	–	–	–	–	–

$\bar{x}\pm$SEM [%]

of physiological forms and a significantly lower percentage of pathological forms of contraction ($P<0.01$).

In transitional cells (Fig. 5B, Table 1) at the onset of pure ischaemia, most sarcomeres are contracted and a smaller proportion is relaxed; over- and hypercontractions both occur very infrequently. During 10 min of pure ischaemia the ratio of physiological to pathological contraction states is about 80 to 20. A 30-min ischaemia leads, on the one hand, to a slight increase in relaxation, to a decrease in contraction and on the other to a drastic increase in pathological contraction states.

In transitional cells, HTK cardioplegia at the onset of ischaemia completely prevents the occurrence of pathological forms of contraction: relaxed and contracted sarcomeres are both seen in about equal amounts. After 30 min, relaxation is found in almost two-thirds, contraction in about one-third; overcontractions, hypercontractions and contraction bands are rare.

In the subendocardial working myocardium (Fig. 5C, Table 1) immediately following aortic cross-clamping there is about one-third relaxation, one-half contraction, some overcontraction and very few hypercontraction. Ten minutes of pure ischaemia leads to a decrease in relaxation, no change in contraction and to a marked increase in pathological contractions, especially of hypercontractions and contraction bands. After 30 min of pure ischaemia, the relaxation of the sarcomeres has increased to almost two-thirds. The cells are still contracted and overcontracted to about one-third. However, we also find here hypercontractions and contraction bands.

Immediately following HTK cardioplegia the superficial working myocardium is relaxed and contracted in almost one-half and seldom overcontracted. Thirty minutes of ischaemia brings about an increase in relaxation and a decrease in contraction, overcontractions and hypercontractions occur very seldom.

The sarcomeres of the intramural working myocardium (Fig. 5D, Table 1) immediately following initiation of pure ischaemia are mostly contracted, to a lesser extent overcontracted or hypercontracted; a lesser proportion is relaxed. Ten minutes of pure ischaemia leads to a remarkable increase of relaxation, and a relative constancy of contraction; pathological contraction states are not found. Following an ischaemic stress of 30 min (25° C), one-third of the intramural heart muscle cells is relaxed and two-thirds are contracted; only a very small fraction are overcontracted.

At the onset of ischaemia after HTK cardioplegia, relaxation of sarcomeres is found in the intramural working myocardium in almost one-third, contraction in about two-thirds, and overcontraction in a few cases. After 10 min of ischaemia relaxation occurs in two-thirds and contraction in one-third of the cells. After

30 min of ischaemia the relaxation has significantly increased to more than two-thirds ($P<0.02$) and the contraction has decreased to less than one-third ($P<0.01$); overcontractions are found very seldom.

Thus, the intramural working myocardium, in contrast to the subendocardial layers, shows appreciable pathological contraction states only at the beginning of pure ischaemia (Fig. 5D). These are significantly reduced by HTK cardioplegia ($P<0.02$). In the different subendocardial cell types (Fig. 1), the peak of pathological contraction states in the course of pure ischaemia is reached later and later the nearer the different cells lie to the endocardium (Fig. 5A–C).

Discussion

The striking ultrastructural alterations of the Purkinje fibres and of the transitional cells during 30 min of pure ischaemia are not only pronounced compared with the subendocardial working myocardium, but also compared with the intramural working myocardium (Figs. 1–4; cf. Schmiedl et al. 1990a; Schnabel et al. 1990a). The differences in the contraction state of the sarcomeres are most obvious (Figs. 5A–D, Table 1). Our definitions of the different contraction states are in accordance with those of other authors (Bing and Fishbein 1979; Berry et al. 1981; Vanderwee et al. 1981; Ganote 1983; Arnold et al. 1985; Karch and Billingham 1985; Todd et al. 1985; Armiger and Smeeton 1986; Vander Heide et al. 1986; Baroldi 1988). Most important is the electron microscopical differentiation between hypercontractions and contraction bands: in contraction bands the actin-myosin clumping brings about ruptures of the contractile system, not seen in hypercontraction (Karch and Billingham 1985; Armiger and Smeeton 1986; Vander Heide et al. 1986; Mihatsch 1988). Light microscopy cannot clearly distinguish these stages (Ashraf and Rahamathulla 1989). The alterations of the contractile system are regarded as a very sensitive indicator of damage to heart muscle cells, which initially occurs inhomogeneously as a focal event (Bing and Fishbein 1979; Todd et al. 1985; Armiger and Smeeton 1986; Schnabel et al. 1987). Thus, different attempts were made to quantify defined lesions of the contractile system (Berry et al. 1981; Todd et al. 1985; Vander Heide et al. 1986; Miyazaki et al. 1987; Ashraf and Rahamatulla 1989). In this study we were able to describe quantitatively, for the first time, the entire contraction state of the different cell types and layers in the subendocardial region of the left ventricle during global ischaemia (other data are published elsewhere – Schnabel et al. 1990a, b, 1991).

The changes in the contraction state of the intramural working myocardium during ischaemia can be explained with the help of the biochemical results (Fig. 5D; Schnabel et al. 1990a, b). Overcontractions are found only at the beginning of pure ischaemia (Schmiedl et al. 1989, 1990b) in combination with high creatine phosphate (CP) and adenosine triphosphate (ATP) concentrations, which apparently allow the small samples of non-suspended myocardium to react to the chemical and osmotic stimulus of the fixation solution with a strong contraction or even an overcontraction (Billingham 1983; Schnabel et al. 1987, 1990b).

Relatively high concentrations of CP and ATP especially in combination with the effects of acidosis and other metabolic changes during ischaemia (Elias et al. 1981) may be responsible for overcontractions as a reaction to the fixative. They could not, however, be responsible for the serious contraction bands in Purkinje fibres and transitional cells, particularly after 30 min of pure ischaemia (Figs. 1C, 5A, B; Table 1). There may be a connection with a particular sensitivity of Purkinje fibres to acidosis and/or glycolytic products (Neely and Grotyohann 1984; Adamantidis et al. 1986). Interstitially, a pH value of about 6.0 is reached after 30 min of pure ischaemia (Schnabel et al. 1990a, b). Apart from affecting the ultrastructure of the capillary endothelium (Bretschneider et al. 1983; Schnabel et al. 1987) this pH may increase the permeability of the endothelial barrier for certain toxic substances.

The pathological overcontractions of subendocardial Purkinje fibres in the course of pure ischaemia observed at an overall tissue ATP concentration of 20 $\mu mol/g_{DW}$ of left ventricular myocardium (corresponding to t-ATP; Schnabel et al. 1990a, b) described here are a striking but not an exceptional result: the reaction pattern for Purkinje fibres shown at t-ATP during pure ischaemia (Figs. 1A, 2C, 5A, B; Ramsauer et al. 1989) and following St. Thomas cardioplegia (Schnabel et al. 1988a, b, 1990b) reminds us very much of the description of the findings of Trofinov in traumatic shock in man (quoted from Doerr 1957). It would seem feasible that Purkinje fibres, because of the differences of mechanical stress and myofibrillar organization compared with the working myocardium, would react particularly strongly to contractile stimuli even in the presence of relaxation in the working myocardium (Thornell et al. 1976; Canale et al. 1983; Schnabel et al. 1988a).

For those Purkinje fibres which develop contraction bands as a sign of irreversible ischaemic damage (Ganote 1983), a particular noxa must be assumed, since "cutting edge lesions" (Todd et al. 1985; Armiger and Smeeton 1986; Baroldi 1988) can be ruled out because of the dissection technique and by very careful presorting of the sections. According to Vander Heide et al. (1986) the appearance of contraction bands can be based on two differing circumstances in heart muscle cells, high ATP concentration and calcium overloading, and very low ATP concentration and slight mitochondrial ATP production which result in the aggravation of rigor complexes until contraction bands develop. The high degree of alteration in the mitochondria in the affected cells (swelling of the mitochondria, loss of matrix and cristae fragmentation even to cristolysis) seem to point to loss of ATP (Schmiedl et al. 1990a; Schnabel et al. 1990b) in our study. Lurie et al. (1987) were able to show that a decrease in the tissue CP and ATP concentrations in the subendocardial working myocardium during superfusion with an oxygenated Tyrode's solution begins at a distance of more than 200 μm from the endocardium.

Oxygen is supplied to the subendocardial cell layers by the process of diffusion. The effectiveness of this would depend on the oxygen consumption of the different cells (Bretschneider 1961; Gebhard and Bretschneider 1989). Therefore, it is quite possible that a mitochondrial "residual aerobiosis" might exist. It is striking that this partial aerobiosis of the marginal layers does not appear to be advantageous for the ischaemic tolerance of these structures (Figs. 1–4).

Major causative factors for the lesions described include catecholamines, calcium and oxygen (Singal et al. 1983; Arnold et al. 1985; Karch and Billingham 1985; Schnabel et al. 1990b). The lesion is often described following clinical and experimental situations where elevated endogenous or exogenous catecholamine levels occur, such as in haemorrhagic shock, cardiovascular shock, hypoxia, ischaemia, cerebral damage, brain death (Arnold et al. 1985; Karch and Billingham 1985; Todd et al. 1985; Novitzky et al. 1988). Catecholamines are also released in great amounts as a reaction to continuously falling blood pressure during inflow occlusion and following cross-clamping of the aorta during the earlier phases of ischaemia (Schömig et al. 1984). They produce typical contraction bands in certain localizations: primarily, in the endocardial third of the free wall of the left ventricle and in the apical position (Arnold et al. 1985; Todd et al. 1985). Our samples during the 1st h of global ischaemia were taken at these sites. Armiger and Smeeton (1986) describe small foci of contraction bands in the subendocardial position which even occur after sudden cardiac death without alterations of coronary vessels (cf. Todd et al. 1985). A catecholamine induced calcium overload can be initiated by pertubation of the sarcolemma (Dhalla et al. 1982; Elz and Nayler 1988; Ashraf and Rahamathulla 1989; Yunge et al. 1989).

We suggest that the contraction bands which appear in the subendocardium during ischaemia can be understood in the sense of a developing contraction band necrosis (Ganote 1983; Armiger and Smeeton 1986). Contraction banding is an energy-dependent process which occurs before fixation as a sign of irreversible injury (Vander Heide 1986; Mihatsch 1988). The irreversibility of the contraction bands in Purkinje fibres has also been demonstrated by perfusion fixation (Schnabel et al. 1988c, 1990b). The bands may bring about a local block or delay of conduction forming the basis for ventricular arrhythmias (Sandoe and Sigurd 1984; Gettes 1986; Hearse 1988).

Acknowledgements. This work was supported by the Deutsche Forschungsgemeinschaft, SFB 330 – Organprotektion Göttingen. The authors are indebted to Prof. G. Mall, (Pathologisches Institut der Universität Heidelberg, FRG) and D.H. Hunneman, PhD for encouraging discussions. We gratefully acknowledge the skilful technical support of E. Bürger, R. Dohrmann, I. Markmann, and the great expertise in the electron microscopy of E. Ehbrecht and A. Gerken. We are especially grateful to C. Maelicke, BSc for converting our cumbersome German into readable English, to E. Neumeyer for the preparation of the figures and to A. Dawe for her continuing friendliness while preparing the manuscript. Portions of this paper will be presented as parts of the medical theses of B. Ramsauer und U. Bartels.

References

Adamantidis MM, Caron JF, Dupuis BA (1986) Triggered activity induced by combined mild hypoxia and acidosis in guinea-pig Purkinje-fibers. J Mol Cell Cardiol 18:1287–1299

Allen DG, Orchard CH (1987) Myocardial contractile function during ischemia and hypoxia. Circ Res 60:153–168

Anderson PG, Bischop SP, Digerness SB (1987) Transmural progression of morphologic changes during ischemic contracture and reperfusion in the normal and hypertrophied rat heart. Am J Pathol 129:152–167

Appelbaum A, Kirklin JW, Tracey W, Blackstone EH (1983) Experimental studies on ischemic contracture. In: Kaplitt MJ, Borman JB (eds) Concepts and controversies in cardiovascular surgery. Appleton-Century-Crofts, Norwalk, Conn, pp 165–173

Armiger LC, Smeeton WMI (1986) Contraction-band necrosis: patterns of distribution in the myocardium and their diagnostic usefullness in sudden cardiac death. Pathology 18:289–295

Armiger LC, Urthaler F, James TN (1979) Morphological changes in the right ventricular septomarginal trabecula (false tendon) during maturation and ageing in the dog heart. J Anat 129:805–817

Arnold G, Kaiser C, Fischer R (1985) Myofibrillar degeneration – a common type of myocardial lesion and its selective identification by a modified Luxol Fast Blue stain. Pathol Res Pract 180:405–415

Ashraf M, Rahamathulla PM (1989) Beneficial effects of low doses of ethanol on reoxygenation injury following anoxia in rat hearts. Basic Res Cardiol 84:378–387

Berry CL, van der Walt J, Wyse R (1981) Sarcomere relaxation and ischaemic myocardial injury. Virchows Arch [A] 390:205–210

Baroldi G (1988) Anatomy and quantification of myocardial cell death. Methods Archiev Exp Pathol 13:87–113

Billingham ME (1983) The role of endomyocardial biopsy in the diagnosis and treatment of heart disease. In: Silver MD (ed) Cardiovascular pathology, vol 2. Churchill Livingstone, New York, pp 1205–1224

Bing OL, Fischbein MC (1979) Mechanical and structural correlates of contracture by metabolic blockade in cardiac muscle from the rat. Circ Res 45:298–308

Bolli R, Patel BS, Hartley CJ, Thornby JI, Jerudi MO, Roberts R (1989) Nonuniform transmural recovery of contractile function in stunned myocardium. Am J Physiol 257:H375–F385

Bretschneider HJ (1961) Sauerstoffbedarf und -versorgung des Herzmuskels. Verh Dtsch Ges Kreisl-Forsch 27:32–59

Bretschneider HJ, Gebhard MM, Gersing E, Preusse CJ, Schnabel PhA (1983) Recent advances for myocardial protection. In: Kaplitt MJ, Borman JB (eds) Concepts and controversies in cardiovascular surgery. Appleton-Century-Crofts, Norwalk, Conn, pp 174–185

Canale E, Campbell GR, Uehara Y, Fujiwara T, Smolich JJ (1983) Sheep cardiac Purkinje fibers: configurational changes during the cardiac cycle. Cell Tissue Res 232:97–110

Dhalla NS, Pierce GN, Panagia V, Singal PK, Beamish RE (1982) Calcium movements in relation to heart function. Basic Res Cardiol 77:117–139

Doerr W (1957) Die Morphologie des Reizleitungssystems, ihre Orthologie und Pathologie. In: Spang K (ed) Rhythmusstörungen des Herzens. Thieme, Stuttgart, pp 1–46

Elias EA, Elias RA, De Vries GP, Meijer AEFH (1982) Early and late changes in the metabolic pattern of the working myocardial fibres and Purkinje fibres of the human heart under ischaemic and inflammatory conditions: an enzyme histochemical study. Histochem J 14:445–459

Elz JS, Nayler WG (1988) Contractile activity and reperfusion-induced calcium gain after ischemia in the isolated rat heart. Lab Invest 58:653–659

Friedman PL, Fenoglio JJ, Wit AL (1975) Time course for reversal of electrophysiological and ultrastructural abnormalities in su-

bendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. Circ Res 36:127–144
Ganote CE (1983) Contraction band necrosis and irreversible myocardial injury. J Mol Cell Cardiol 15:67–73
Gebhard MM, Bretschneider HJ (1989) Myocardial protection. Curr Opin Cardiol 4:803–806
Gettes LS (1986) Effects of ischemia on cardiac electrophysiology. In: Fozzard HA (ed) The heart and cardiovascular system. Raven Press, New York, pp 1317–1341
Hearse DJ (1988) The protection of the ischemic myocardium: surgical success vs clinical failure. Prog Cardiovasc Dis 30:381–402
Karch SB, Billingham ME (1985) Myocardial contraction bands revisited. Hum pathol 17:9–13
Lowe JE, Cummings RG, Adams DH, Hullryde EA (1983) Evidence that ischemic cell death begins in the subendocardium independent of variation in collateral flow or wall tension. Circulation 68:190–202
Lurie KG, Argentieri TM, Sheldon J, Frame LH, Matschinsky FM (1987) Metabolism and electrophysiology in subendocardial Purkinje fibers after infarction. Am J Physiol 253:H662–H670
Mihatsch MJ (1988) Die Morphologie des Myokardinfarkts. Schweiz Med Wochenschr 118:1688–1691
Miyazaki S, Fujiwara H, Onodera T, Kihara Y, Matsuda M, Wu D-J, Nakamura Y, Kumada T, Sasayama S, Kawai C, Hamashima Y (1987) Quantitative analysis of contraction band and coagulation necrosis after ischemia and reperfusion in the porcine heart. Circulation 75:1074–1082
Neely JR, Grotyohann LW (1984) Role of glycolytic products in damage to ischemic myocardium. Circ Res 55:816–824
Novitzky D, Rose AG, Cooper DKC (1988) Injury of myocardial conduction tissue and coronary artery smooth muscle following brain death in the baboon. Transplantation 45:964–966
Preusse CJ, Schulte HD, Bircks W (1987) High volume cardioplegia. Ann Chir et Gyn 76:39–45
Ramsauer B, Schnabel PhA, Schmiedl A, Bartels U, Gebhard MM, Richter J, Bretschneider HJ (1989) Schädigungsmuster von Purkinje-Fasern und Arbeitsmyokard nach Kardioplegie und Ischämie. Verh Dtsch Ges Pathol 73:511
Richter J, Schnabel PhA, Pflug M, Gebhard MM, Bretschneider HJ (1986) Elektronenmikroskopische Untersuchungen an Purkinje-Fasern bei globaler Ischämie unter Myokardprotektion. Verh Anat Ges 80:567–570
Sandoe E, Sigurd B (1984) Arrhythmia. Fachmed, St. Gallen
Schmiedl A, Schnabel PhA, Gebhard MM, Richter J, Bretschneider HJ (1989) Einfluß der Vorbehandlung auf den Kontraktionszustand und die Ultrastruktur von Myokardbiopsien. Z Kardiol 78 [Suppl 4]:57
Schmiedl A, Schnabel PhA, Mall G, Gebhard MM, Hunneman DH, Richter J, Bretschneider HJ (1990a) The surface to volume ratio of mitochondria, a suitable parameter for evaluating mitochondrial swelling – correlations during the course of myocardial global ischaemia. Virchows Arch [A] 416:305–315
Schmiedl A, Schnabel PhA, Haasis G, Mall G, Gebhard MM, Richter J, Bretschneider HJ (1990b) Influence of pretreatment on interstitial and intracellular space of canine left-ventricular myocardium. Acta Anat 138:175–181
Schmiedl A, Schnabel PhA, Gebhard MM, Richter J, Bretschneider HJ (1990c) Influence of the method of cardiac arrest and of the ATP-concentration on the contraction state of working myocardium during global ischemia. Pflügers Arch 415 [Suppl 1]:R60
Schnabel PhA, Gebhard MM, Pomykaj T, Schmiedl A, Preusse CJ, Richter J, Bretschneider HJ (1987) Myocardial protection: left ventricular ultrastructure after different forms of cardiac arrest. Thorac Cardiovasc Surgeon 35:148–156
Schnabel PhA, Gebhard MM, Richter J, Schmiedl A, Bretschneider HJ (1988a) Feinstruktur subendokardialer Purkinje-Fasern während und nach Ischämie: Einfluß unterschiedlicher kardioplegischer Lösungen. Z Herz-Thorax-Gefäßchir 2:54–61
Schnabel PhA, Clavien H-J, Kehrer G, Ramsauer B, Schmiedl A, Gebhard MM, Richter J, Bretschneider HJ (1988b) Strukturelle Ischämietoleranz subendokardialer Purkinje-Fasern im Vergleich zum Arbeitsmyokard. Verh Dtsch Ges Pathol 72:581
Schnabel PhA, Gebhard MM, Pflug M, Richter J, Schmiedl A, Bretschneider HJ (1988c) Reversibilität feinstruktureller Veränderungen der Purkinje-Fasern nach Kardioplegie und globaler Ischämie. Z Kardiol 77 [Suppl 1]:95
Schnabel PhA, Richter J, Gebhard MM, Mall G, Schmiedl A, Clavien H-J, Bretschneider HJ (1990a) Ultrastructural effects induced by global ischaemia on the AV node compared with the working myocardium: a qualitative and morphometric investigation on the canine heart. Virchows Arch [A] 416:317–328
Schnabel PhA, Richter J, Schmiedl A, Ramsauer B, Bach F, Haasis G, Gebhard MM, Mall G, Bretschneider HJ (1990b) Schädigungsmuster und Strukturprotektion subendokardialer Purkinje-Fasern bei globaler Myokardischämie. In: Ganten D, Mall G (eds) Herz-Kreislaufregulation, Organprotektion und Organschäden. Schattauer, Stuttgart (in press)
Schnabel PhA, Richter J, Schmiedl A, Ramsauer B, Bartels U, Gebhard MM, Mall G, Bretschneider HJ (1991) The ultrastructural effects of global ischaemia on Purkinje fibres compared with working myocardium: a qualitative and morphometric investigation on the canine heart. Virchows Arch [A] (to appear)
Schömig A, Dart AM, Dietz R, Mayer E, Kübler W (1984) Release of endogeneous catecholamines in the ischemic myocardium of the rat. Circ Res 55:689–701
Singal PK, Beamish RE, Dhalla NS (1983) Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. Adv Exp Med Biol 161:391–401
Thornell L-E, Sjöstrom M, Andersson K-E (1976) The relationship between mechanical stress and myofibrillar organization in heart Purkinje fibres. J Mol Cell Cardiol 8:689–695
Todd GL, Baroldi G, Pieper GM, Clayton TC, Eliot RS (1985) Experimental catecholamine-induced myocardial necrosis. I. Morphology, quantification and regional distribution of acute contraction band lesions. J Mol Cell Cardiol 17:317–338
Vander Heide RS, Angelo JP, Altschuld RA, Ganote CE (1986) Energy dependence of contraction band formation in perfused hearts and isolated adult myocytes. Am J Pathol 125:55–68
Vanderwee MA, Humphrey SM, Gavin JB, Armiger LC (1981) Changes in the contractile state, fine structure and metabolism of cardiac muscle cells during the development of rigor mortis. Virchows Arch [B] 35:159–167
Weibel ER (1979) Stereological methods, vol 1. Academic Press, New York
Yunge W, Bruneval P, Cokay MS, Berry B, Peters H, Poulsen R, Hüttner I (1989) Pertubation of the sarcolemmal membrane in isoproterenol-induced myocardial injury of the rat. Am J Pathol 134:171–185