Communication

Microcomputer-assisted determination of regional myocardial function

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1 Introduction

One of the important targets of experimental research on myocardial ischaemia is the evaluation of therapeutic interventions designed to minimise the myocardial dysfunction induced by the ischaemia. Until recently, these types of experiments relied heavily on post-mortem evaluations of irreversibly damaged myocardium. Such methods can be useful if the duration of ischaemia is sufficiently long and injurious to produce substantial myocardial infarction. However, it is now known that even short durations of regional ischaemia (10–15 min and less) can induce significant, and sometimes sustained myocardial dysfunction, without producing a measurable infarct. This myocardial 'stunning' phenomenon is the subject of considerable current research. To gain insight into the mechanisms responsible and to test intervention strategies aimed at diminishing the resultant dysfunction, it is necessary to monitor myocardial function continuously before, during and following the ischaemic insult.

Indices of regional myocardial function (RMF) (e.g. segment shortening or wall thickening) give considerably more information about the state of the myocardial tissue as compared to indices of global myocardial function (e.g. left ventricular pressure). Among the many indices of RMF, one of the most effective is the determination of the systolic wall thickening with the use of an epicardial Doppler probe (Hartley *et al.*, 1983).

In this communication we describe and show pertinent results of a method whereby RMF data can be acquired by a microcomputer online during experimental procedures, thereby providing an instantaneous monitoring of the time course of RMF. This is a major advantage in that it allows the experimenter to determine the presence of trends (e.g. steady state) and other dynamic features of the changing RMF, at times when appropriate experimental or therapeutic interventions should be made. In addition, the method has other advantages. It eliminates tedious, post-experiment manual methods of data extraction from chart recorder waveforms. The data, being automatically stored in digital form, provide the necessary database to perform analysis and statistical procedures easily. Statistical analysis can even be performed to determine the normally occurring and variant beat-to-beat variations in RMF. This type of analysis would not be feasible using conventional 'manual' methods.

It turns out that one of the major problems associated with automatic determination of RMF (using any method) is the automatic determination of time points corresponding to the end of diastole and the end of systole. In this paper we also give details of our approach to locating these time points.

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2 Methods

2.1 *Experimental setup*

The method to be described was developed to evaluate RMF in rabbit hearts. The choice of the rabbit model was based on planned therapeutic interventions, which are not relevant to the present report. The experimental protocol, in short, consisted of anaesthetising the rabbit (New Zealand white, 3·5–4·5 kg) with pentobarbitol (30 mg kg^{-1} over initial 30 min, 3 mg kg^{-1} h^{-1} throughout the experiment), intubating it, and exposing its heart via midline sternotomy. Next, the rabbits were instrumented with an intraventricular high-fidelity pressure sensing catheter (Millar TC-510 catheter, Millar MPC-500 controller) placed through a puncture at the apex of the heart. Two epicardial Doppler probes (10 MHz, 4 mm diameter disk shaped with a defocusing epoxy lens on one side, Crystal Biotech) were glued (using cynoacrylate) in the risk and non-risk regions of the heart. The risk region was the area to be rendered ischaemic, and the non-risk region was an area outside of the risk region but still on the left ventricular free wall. Care was taken to glue only the fabric patch attached to the piezoelectric crystals, and not the crystals themselves. Glueing at three points assured the movement of the crystal parallel to the epicardial surface. Aortic blood pressure was monitored with a fluid-filled catheter (14 gauge cut to 35 mm) introduced into the thoracic aorta via the left carotid artery. Standard limb lead electrodes were connected for the ECG. Core temperature was measured oesophageally. Oxygen saturation was measured using a pulse oximeter (Ohmeda 3740) with the probe located on a shaved section of the rabbit tail.

Fig. 1 illustrates schematically the basic data and signal flow during an experimental protocol. The signals from the transducers on the rabbit go through amplifiers and/or

signal processors, the outputs of which are input to the analogue-to-digital (A/D) convertor. Digitised data are processed and the results are displayed graphically on the computer screen. On the command of the user, the results are printed on a hard-copy device and also stored on disk for further reference. The first derivative of the LV pressure (LV dP/dt) is generated using an analogue differentiator,

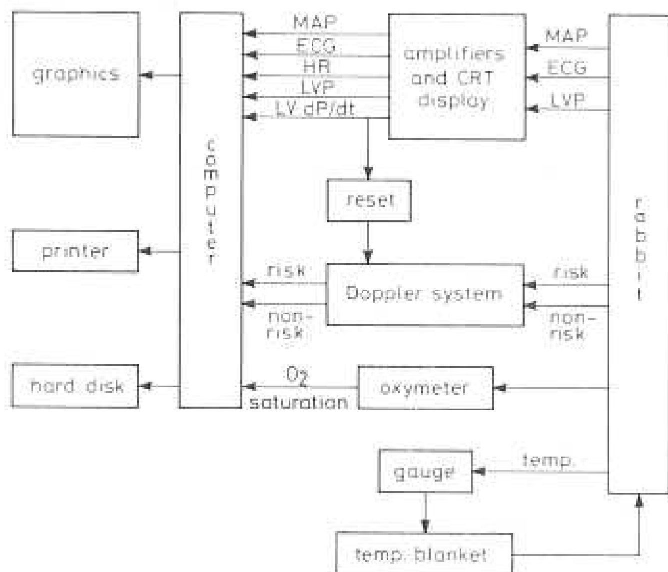

Fig. 1 *Schematic diagram showing the data and signal flow during an experimental protocol. The abbreviations used are: MAP (mean aortic pressure), ECG (electrocardiogram), LVP (left ventricular pressure), LV dP/dt (time derivative of the left ventricular pressure), HR (heart rate), risk (thickening fraction in the risk region), non-risk (thickening fraction in the non-risk region) and temp (temperature)*

which is calibrated before every experiment using a ramp input of known slope. The reason for the analogue rather than digital differentiation is that the analogue signal is needed elsewhere. It is used to set the thickening signal to a predetermined value during the diastole of every heartbeat (further detail in next section).

2.2 *Myocardial function*

Determination of the systolic myocardial thickening fraction (TF) with the use of a single epicardial Doppler probe has been detailed by HARTLEY *et al.* (1983) and validated by ZHU *et al.* (1986). In short, this system (VF1 Doppler System, Crystal Biotech) uses the pulsed Doppler principle (10 MHz ultrasonic frequency and 3·91 kHz pulse repetition frequency) to determine the average velocity of myocardial layers passing through a virtual sample volume. The digital integration of the velocity results in a relative measure of wall thickness. The continuous integration brings about the possibility of signal drift. Accordingly, to be on the safe side, the signal needs to be reset every heartbeat without affecting the actual measurement. As TF is measured during systole, the signal can be reset any time during diastole. A reset pulse at the onset of the R-wave of the ECG is used with dogs, but in open-chest rabbits it was determined that the ECG was too variant to be used reliably for this purpose. To circumvent this problem, an analogue circuit was designed that uses a constant time delay (40 ms) after the detection of the negative peak of the LV dP/dt signal to output the 'reset' pulse. This guarantees that the resetting will occur within the diastolic phase of every heartbeat.

To set the location of the virtual sample volume, the range gate is increased from zero until the pitch of the audio signal (corresponding to the Doppler shift) changes from a heartbeat sound to a blood flow sound (about 3–6 mm from the epicardium). The virtual volume is estimated to be 2 mm^3 for the transducers used in these experiments. Assuming the virtual sample volume is located close to the endocardium at end-diastole, one can approximate TF with the following formula:

$$TF = 100 \times (e^{ME/R} - 1)$$

where R is the range gate in mm, or, in other words, it is the location of the virtual sample volume with respect to the epicardial surface. ME (maximum excursion) is defined as the difference (in mm) of the relative wall thickness between end-diastole and the earlier of the two events; end-systole or peak of the relative wall thickness. HARTLEY *et al.* (1983), who derived the formula theoretically, subtracted the amount of wall thinning, if present, from the above definition of ME. We did not follow their path; instead we kept track of the amount and duration of thinning as two separate variables. The relationship between the thinning and thickening in the rabbit will be part of a future paper.

Accuracy of TF, aside from the limitations of the hardware, depends on the determination of R and ME. As R is usually held constant over the whole experimental protocol, the determination of ME becomes very important. In the next section, we will report the methods used to locate the start and end of systole, which have direct influence on the accuracy of ME.

2.3 *End-diastole and end-systole*

The LV dP/dt signal was used to determine simply and reliably both the end-diastolic and the end-systolic points. LV dP/dt is commonly available in invasive protocols and, due to the simplistic nature of the waveform, it requires a minimum of signal processing as opposed to noninvasive signals such as ECG or the phonocardiogram (PCG). The major drawback of the LV dP/dt signal is the amplification of high-frequency amplifier noise due to differentiation.

The normal LV dP/dt waveform has one positive and one negative peak for a given heartbeat. The zero-crossing of the LV dP/dt before the positive peak (OSAKADA *et al.*, 1983) or the rapid rise of the LV pressure (BOLLI *et al.*, 1989) have been widely accepted as the end-diastolic time points. In an LV dP/dt waveform, with no noise, the former definition would be most representative of end of diastole as it marks the start of the contraction in the ventricle. Our preliminary experiments showed that the zero-crossing could not be detected reliably in the presence of noise. Accordingly, we chose the time when the LV dP/dt signal reaches 10 per cent of the amplitude of that specific beat (Fig. 2). This point in time (10 per cent crossing) could be determined much more reliably as the slope of the LV dP/dt signal was higher than that at the zero-crossing. Accordingly the effect of the noise was reduced. The difference in time from the zero-crossing to the 10 per cent crossing was about 2 per cent of the systolic duration (a representative systolic interval was 130 ms).

In our digital system, where all data samples within a window are stored in memory, the end-diastolic point is determined backwards; first the positive peak of the LV dP/dt is detected, and then the algorithm goes backwards in time until the 10 per cent crossing is reached, which is flagged as end of diastole. The possible bias error introduced due to the definition of end-diastole was considered negligible for a sampling rate of 600 Hz per channel, which spaces the data points by 1·7 ms.

The point of end-systole (closure of the aortic valve)

using the LV dP/dt signal has not been defined very consistently. Some researchers (BOLLI *et al.* 1989) use the negative peak, whereas others (SASAYAMA *et al.*, 1981) use 20 ms before the negative peak in the dog. Most of the time, it is obvious that the negative peak of LV dP/dt is already within the isovolumic relaxation phase, but it is not trivial to determine the start of the phase with respect to the negative peak. We have reported before (CIDECIYAN *et al.*, 1989; CIDECIYAN, 1988) that under the majority of experimental conditions the exact definition of the end-systole (in the range between 0 and 20 ms before the negative peak of LV dP/dt) has a minimal effect on the calculated TF. Accordingly, for this communication, we used 20 ms before the negative peak of the LV dP/dt signal as the definition of the end-systole (Fig. 2).

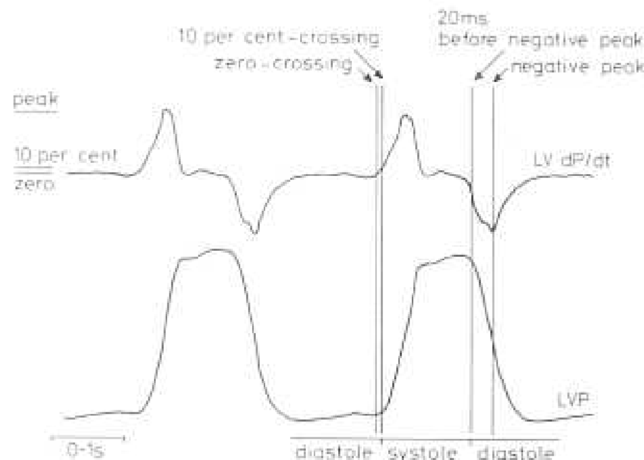

Fig. 2 *Actual waveforms of left ventricular pressure (LVP) and its time derivative (LV dP/dt). The time points corresponding to the zero-crossing and the 10 per cent crossing of the LV dP/dt signal are marked as well as the negative peak LV dP/dt and 20 ms before the negative peak. The regions corresponding to our definitions of diastole and systole are also shown.*

2.4 *Implementation*

The definitions mentioned in the previous sections were coded in 'QuickBasic' (version 4.5, Microsoft Corp.) and combined with code for data acquisition and a user interface. The software ran on an IBM PC/AT microcomputer equipped with an eight-channel A/D convertor board (Dash-16, Metrabyte Corp.). The program not only calculated the RMF at two separate regions of the left ventricle simultaneously, but also displayed graphically the signals acquired and the locations of the end-diastole and end-systole picked by the computer (Fig. 3). Upon command from the user, it also plotted the evolution of the data during the whole experimental protocol, which was very useful in qualitative determination of certain trends during the experimental protocol.

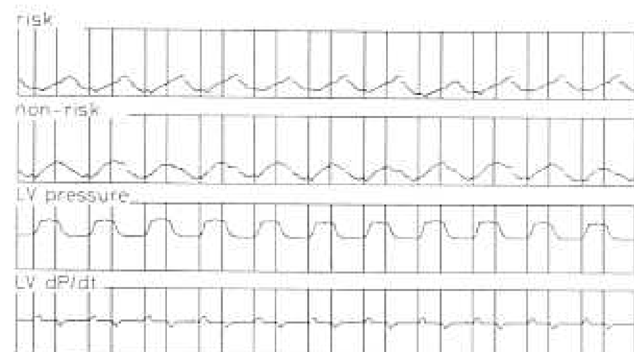

Fig. 3 *Hard copy of a representative computer screen during an experiment. Digitised values of four signals, risk and non-risk wall thicknesses, LV pressure and LV dP/dt within a 3 s window are displayed. The computer-determined end-diastolic and end-systolic locations are marked with vertical lines for every heartbeat. The discontinuities during diastole in risk and non-risk signals are due to automatic resetting of the signals to prevent signal drift*

A total of four signals were sampled with a sampling rate of 600 Hz per channel. They were: TF in risk and non-risk regions, LV pressure and LV dP/dt. The A/D convertor used a multiplexer so that channel-to-channel delay was 0·42 ms. Systemic variables: mean aortic pressure, heart rate and O_2 saturation, were also sampled at a much slower rate (1 Hz). They were very useful in categorising the overall state of the animal during the protocol.

To minimise the effects of breathing, the variables were averaged for the consecutive heartbeats in a 3 s window (10 heartbeats for a representative heart rate of 200 beats min^{-1}). The determination of all measurements for a 3 s window lasted about 35 s, but this was not a limitation during our long protocol, as there never was the need to acquire data with less than 5 min intervals.

3 Experimental results

Some relevant results obtained using the microcomputer-assisted method are shown in Figs. 4 and 5. Fig. 4 plots the evolution of TF in the rabbit, before, during and after a 15 min occlusion of a major coronary artery on the left side of the heart (corresponding to the LAD in the dog). It can be seen that TF in the non-risk region stays relatively stable during the experimental protocol, whereas TF in the risk region drops to below zero (thinning or dyskinesia) during the occlusion and returns to baseline within one hour of reperfusion.

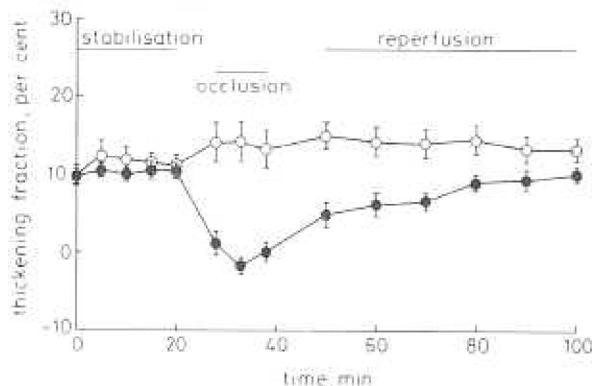

Fig. 4 *Thickening fraction in the risk and non-risk regions of the myocardium of rabbits ($n = 8$) during 15 min occlusion, 1 h reperfusion experiments. The error bars represent the standard error*
●—● *risk*; ○—○ *non-risk*

Fig. 5 on the other hand characterises a different TF evolution curve, which was accumulated during 45 min occlusion, 6 h reperfusion studies. The artery used was the same as the 15 min occlusion study. Similarly to the previous protocol, TF in the non-risk region stays stable at control levels throughout the experiment. On the other hand, TF in the risk region drops to below zero function right after the occlusion, and stays around zero (akinesis) for the rest of the experiment.

The fact that TF in the risk region never recovers during the 6 h reperfusion suggests that a 45 min ischaemic period results in permanent tissue damage in the open-chest rabbit. In these animals, the amount of tissue that was rendered ischaemic (risk) was determined post-mortem by injecting fluorescent microspheres into the vascular bed

supplied by the artery occluded. After freezing, the heart was sliced at 4 mm intervals and the fluorescent area on every slice was measured. To determine the areas of infarction, the heart slices were incubated in Nitroblue Tetrazolium, which stains the viable tissue blue. The areas and slice thicknesses were used to estimate the volumes of risk and infarct, assuming a conical shape for the heart. The amount of infarct in the animals with the 45 min ischaemia was found to be 46·8 ± 2·7 per cent of the risk volume, whereas there was no detectable infarction among the animals of the 15 min ischaemia.

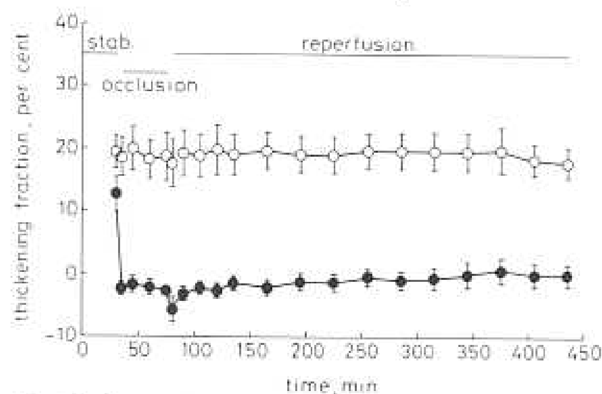

Fig. 5 *Thickening fraction in the risk and non-risk regions of the myocardium of rabbits (n = 12) during 45 min occlusion, 6 h reperfusion experiments. The abbreviation 'stab.' stands for the 30 min stabilisation period. The control data point is the last datum taken before the occlusion. The error bars represent the standard error*
● — ● *risk*; ○ — ○ *non-risk*

4 Discussion and conclusion

Measurement of RMF can be very useful in gaining insight into the mechanisms of the myocardial 'stunning' phenomenon. To accomplish this measurement, the usual practice has been to record the data initially on a chart recorder during the experiment. Later, the relevant time points are manually determined, usually by a technician, and the necessary measurements are made. Eventually, the generated data are entered into a computer for further analysis.

In this paper, we have presented the use of a microcomputer to determine RMF during an experimental protocol. The side-effect of this process is that the raw data are automatically stored in the computer ready for further processing. The assistance of a microcomputer also eliminates the loss of data due to a variety of human errors, especially during long experimental protocols. The fact that RMF values are available throughout the experiment allows the experimenter to take necessary measures, when unusual fluctuations occur; as opposed to finding out that the experiment is not valid after the protocol is finished and the data have been analysed.

Our computer-assisted method was intentionally not made completely automatic. It required the presence of a trained observer who understood the process by which the software determined the relevant timing points and who also had knowledge about the possibilities of errors. The observer would have to hit a key to accept the data generated and graphically displayed by the computer. This process guaranteed to match the accuracy of the human as a minimum. Our experience with the method showed that once certain variables were adjusted to the particular animal and all signals calibrated at the start of an experiment, the required interference by the user during an 8–9 h protocol was minimal. Most of the situations requiring user input arose when a reasonable LV dP/dt waveform was not present due to a variety of transient arrhythmias during the first 5 min of the occlusion. These arrhythmias usually involved variations in heart rate and/or changes in the LV dP/dt waveform.

In conclusion, the microcomputer-assisted method has proven to be of great use in saving hundreds of man-hours of tedious work, increasing repeatability, decreasing the number of invalid experiments and keeping the accuracy of the results at least at the same level as the manual method.

It should be added that our method is applicable to other animals, including dogs, using other wall thickening measurements such as 'transit time'.

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