Determination of infarct size in rats with ^{86}rubidium

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Summary. A quick method is presented which allows the determination of infarct sizes within hearts of experimental animals like rats. The method is based on the drop of ^{86}Rb uptake in infarcted hearts, and has been compared with infarct sizes determined morphometrically. The standard deviation of this method is low (6.5%) because an internal standard is used (noninfarcted myocard - usually right ventricle tissue - has been used).

In experimental cardiology, it is often necessary to determine the size of myocardial necrosis to show the grade of protection by drugs[1,2]. Till now morphological methods have been best suited for recognition of myocardial infarction. Unfortunately at least 3 difficulties emerge in the course of morphometric analysis of infarction: 1. The uncertainty of cell death in the margin area of infarction. 2. The rather time-consuming procedure for morphometric infarct-size determination, which makes it difficult to work with a great number of animals or to get results quickly. 3. The impossibility of morphometric infarct-size determination a few h after infarction.

To overcome the difficulty of point 3, at least in part, we tried to find a method for estimation of cell damage 2 h after isoproterenol application to rats by chemical analysis of myocardial sodium and potassium[3]. But, besides other objections arising from this kind of causing cell damage point 2 also held. Therefore we looked for a simple method to overcome the above-mentioned difficulties.

In analogy to the chemical assay of potassium, we have chosen the uptake of radioactive rubidium as an indicator for living cells, because this ion behaves very similarly to potassium in living tissue[4,5].

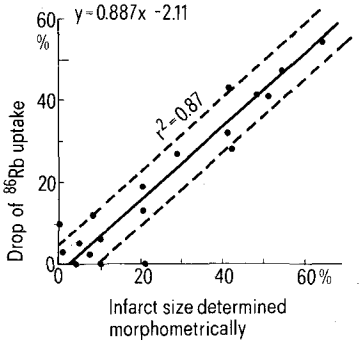
Methods. 24-48 h after ligating the ramus descendens of the left coronary artery, 10 µCi ^{86}Rb (in isotonic saline) were administered to male wistar rats via their tail veins. After 10 min equilibration time, the rats were killed and their hearts quickly removed. The hearts were dissected into right and left ventricle. The latter was further divided into an apical part and a part near the basis. All parts were shortly rinsed with cold saline, blotted on moist filter paper and put into preweighed plastic vials. The measurement of radioactivity was carried out in a well-type counter. Results (expressed in cpm/g of wet tissue) were corrected for self-absorption using ^{86}Rb in different amounts of water (water absorption is nearly equivalent to tissue absorption).

Histochemical reaction and morphometric analysis 26 frozen sections (12.5 µm thick) were prepared from both parts of the left ventricle. To demonstrate infarct sizes, a histochemical reaction for succinate dehydrogenase activity with nitro blue tetrazolium (NBT) was used[6]. Final concentrations: Na-succinate 0.05 M, NBT 0.5 mg/ml, polyvinylpyrrolidon 75 mg/ml, Tris-buffer 0.0125 M pH 7.6. Reaction time: 40 min. The blue colour of NBT-formazan developed in areas without ischemic injuries, but in infarcted areas only traces of formazan granula were seen as the result of a great loss of enzymatic activity. The NBT technique gave the same infarct size as the usual HE staining. Morphometric determination of infarct size was carried out by applying the point counting method[7].

Results and discussion. Assuming that all measured radioactivity is within surviving cells, and the right ventricle is an appropriate reference tissue, infarct size (IS) was calculated according to

$$IS_{\%} = \left[1 - \frac{(\text{cpm/g left ventricle})}{(\text{cpm/g right ventricle})}\right] 100 \qquad (1)$$

(1) relates infarct size to the piece measured. Usually infarct size is calculated as a portion of necrotic tissue from



Regression line of infarct sizes within left ventricles determined morphometrically and by means of ^{86}Rb.
Individual values (basis and apex) and total values of animals indicated with b from the table were utilized for constructing the regression line. Dashed lines = range of standard deviation, SD = ±6.5%.

Comparison of infarct sizes (%) within left ventricles of rats determined morphometrically and by means of ^{86}Rb. All percent numbers relate infarct sizes to the indicated part of left ventricles (basis, apex, total left ventricle). Animals No. 1-6 were killed 48 h, No. 2a, 3a and 5a 36 h, and No. 12b, 24b and 25b 24 h after infarction

No. of animal	Basis		Apex		Total left ventricle	
	Morphometrical	^{86}Rb	Morphometrical	^{86}Rb	Morphometrical	^{86}Rb
1	42	28	48	41	45	34
2	10	6	4	0	7	3
3	54	47	64	54	59	50
5	10	0	21	0	16	0
6	20	19	41	32	31	27
2a	29	27	51	41	39	33
3a	5	5	8	2	7	3
5a	8	12	1	3	5	9
12b	-	-	-	-	0	10
24b	-	-	-	-	20	13
25b	-	-	-	-	41	43

total left ventricle. This may be carried out by calculating the weighed mean

$$IS_{\%} = \frac{\sum (IS_i \cdot W_i)}{\sum W_i} \quad (2)$$

W_i = weight of piece i.

In the table, infarct sizes calculated by the aid of equations (1) and (2) are compared to those determined morphometrically. The figure shows that there is a good correlation between both methods. The coefficient of correlation is 0.94 with a high level of significance ($p > 99.9\%$).

In literature various radioactive methods have been described for detection of ischemic areas and determination of IS[8-10], but until recently[11] no serious effort was made to correlate those with IS determined morphometrically. The SD of the ^{86}Rb values from the regression line (figure) was found to be 6.5%; that is far better than the SD which one may expect by calculating IS from serial serum creatine phosphokinase (CPK) determinations after infarction (20–30%)[12]. If we only take into account the SD of 'normal' myocardial CPK activity, which is important in case of determining IS from myocardial depletion of CPK, a SD of about 16 or 14% may be expected[12,13]. The SD of 6.5% in this work is the consequence of using an internal standard (right ventricle). The limits of sensitivity of our method are determined by methodical errors (5%), which are composed of the errors of weight and cpm determination (including that of cpm correction). If the determination of IS is carried out later than 48 h after infarction, an erroneous result may be obtained, because connective tissue cells begin to grow into the area of infarction. These cells would take up ^{86}Rb, and thereby the calculated IS would be reduced. A systematic error of this method is caused by neglecting the amount of ^{86}Rb which is taken up from infarcted tissue. This fact finds its expression in reducing the coefficient of regression below unity (figure).

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Motor nerve terminal defect following tenotomy[1]

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Summary. Post-tetanic potentiation and the underlying post-tetanic repetition in cat soleus muscle require normal motor nerve terminals. These indices of nerve terminal viability are depressed 10 days and absent 15 days after tenotomy of the soleus muscle.

A muscle detached from its insertion by section of the tendon (tenotomy) is unable to develop significant tension and exhibits signs of chronic disuse. Tenotomy results in alterations in the muscle with concomitant changes in contractile properties[3], its innervation and associated spinal reflexes. The muscle atrophies, discharge from muscle spindles[4-8] and other receptors[9,10] is enhanced and monosynaptic ventral root reflexes and early potentials in the dorsal spinocerebellar tract are augmented[3,11-15].

The relative inactivity of muscles resulting from pinning of the limb causes slight alterations in presynaptic function at the neuromuscular junction[16,17]. However, nerve terminal function following marked muscle inactivity such as that resulting from tenotomy has not been determined using tests which can specifically reveal alterations in motor nerve endings[18-20].

High frequency tetanization of cat soleus nerves conditions the nerve terminals such that subsequent stimuli during the post-tetanic period elicit a burst of repetitive action potentials (post-tetanic repetition, PTR) in the vicinity of the nerve endings[21]. The PTR, which can be recorded in ventral root filaments, causes brief, asynchronous tetanization of the muscle, resulting in potentiation of muscle contractile strength (post-tetanic potentiation, PTP)[22]. PTP is dependent on the frequency of tetanic stimulation; optimum PTP occurs in cat soleus muscle in response to tetanization at 400 Hz. PTP and its underlying PTR are lost prior to transmission failure at the neuromuscular junction when the nerve terminals are subjected to physical[18] or chemical trauma[19,20]. Hence these events serve as sensitive indicators of nerve terminal viability.

Methods. 6 adult cats under pentobarbital anesthesia were tenotomized by disconnecting the Achilles tendon from the calcaneus. The tendon was folded back on itself to prevent reunion by connective tissue. 3 cats were investigated at each of 10 and 15 days following tenotomy. 5 normal cats provided control data. On test days cats were given α-chloralose (80 mg/kg i.v.), an in situ soleus nerve-muscle preparation prepared and the spinal cord exposed by dorsal laminectomy[23]. The soleus tendons were attached to a strain gauge for isometric recording of contractile tension at one-half maximum physiological extension (determined in situ prior to cutting the calcaneus). The soleus nerve was stimulated with supramaximal pulses (0.1 msec duration) at 0.4 Hz except for 10-sec tetanic trains at 25–400 Hz. Ventral roots were subdivided to obtain filaments containing func-