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Role of neurotransmitter release and cyclic AMP-dependent membrane phosphorylation in low voltage myocardial automaticity[1]

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Summary. Low voltage myocardial automaticity (LVA) was investigated by pharmacological modulations of the presynaptic and postsynaptic processes. The sensitivity of LVA both to inhibitor and stimulator of neutrotransmitter release suggests its involvement in LVA genesis. Moreover, LVA is blocked by the inhibition of the cyclic AMP system, supporting the participation of the c-AMP-dependent membrane phosphorylation in calcium-mediated cardiac electrogenesis.

It has been well demonstrated that working myocardium automaticity can be readily induced by a depolarizing current[2-7]. 2 kinds of such automaticity have been revealed; a high voltage, sodium-dependent one, and a low voltage, calcium-dependent one (LVA). The LVA is a most characteristic pattern in mammalian papillary muscle[4-7]. Typically, LVA occurs in the plateau range of the membrane potentials near -45 mV and is thought to be due to repetitive activation of a slow inward current carried mainly by Ca^{2+} in mammalian myocardium[8]. However an outward current activated in the plateau range of potentials may contribute to the LVA undergoing deactivation[9]. LVA has many features in common with pacemaker activity in the nodal cells and the border zone in the myocardial infarction[10]. The causal relationship between neurotransmitter release (NR) and both cited types of automaticity is becoming increasingly clear[11,12]. But the role of endogenous catecholamines for current-induced automaticity remains to be recognized.

Materials and methods. Papillary muscles from the right ventricle of rabbits and guinea-pigs were used. The diameters of the papillary muscles were between 0.85 mm and 1.3 mm. Preparations were pulled through a hole in a tightly fitted wall dividing the tissue chamber into 2 compartments. Current could be made to flow between these compartments through Ag-AgCl electrodes[12]. Tyrode solution of the following composition (in mM): Na^+ 150.8, K^+ 4.0, Ca^{2+} 2.7, Mg^{2+} 1.0, HCO_3^- 12, $H_2PO_4^-$ 1.8, Cl^-148.4, glucose 10, ascorbic acid 0.06,was used. Solutions were gassed with 5% CO_2 + 95% O_2. Temperature was maintained at 36-37 °C, pH 7.4. The conventional microelectrode technique was used. Voltage changes were recorded differentially between an intra- and an extracellular microelectrode located near the separating wall in the compartment containing the tendon-end of the preparation. The tension was recorded isometrically via a Force Displacement Transducer (Type MXB-6). Current, tension and transmembrane voltage were displayed on an oscilloscope and were film-recorded. During the equilibration period (1 h) the muscle was stimulated at a frequency of 1 Hz.

The drugs used were: RMI 123330A, Merrell-National Laboratories, Cincinnati, USA; Tolbutamide - Chugai Pharmaceutical Co. Ltd. Tokyo; Oxymetazoline hydrochloride, Alprenolol, Tyramine hydrochloride - Merck, Darmstadt.

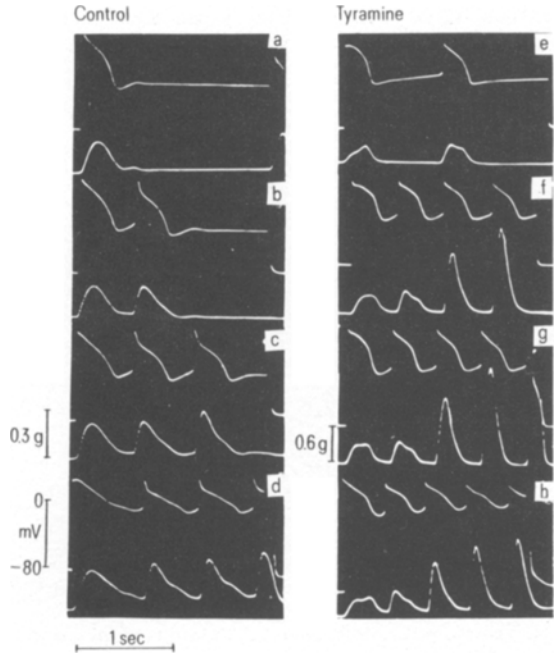

Fig. 1. Tyramine (10^{-4} M)-potentiated repetitive activity (the upper traces) and isometric contraction (the lower traces) in guinea-pig papillary muscle. 15 min after drug administration. Current intensities were: *a* $2.6 \cdot 10^{-5}$ A, *b* $3.1 \cdot 10^{-5}$ A, *c* $3.25 \cdot 10^{-5}$ A, *d* $3.3 \cdot 10^{-5}$ A, *e* $0.8 \cdot 10^{-5}$ A, *f* $2.8 \cdot 10^{-5}$ A, *g* $3.1 \cdot 10^{-5}$ A, *h* $4.8 \cdot 10^{-5}$ A. In this and other figures repetitive firings were induced by 2 sec current pulses increased from top to bottom. Each record was separated by a quiescent period 1 min.

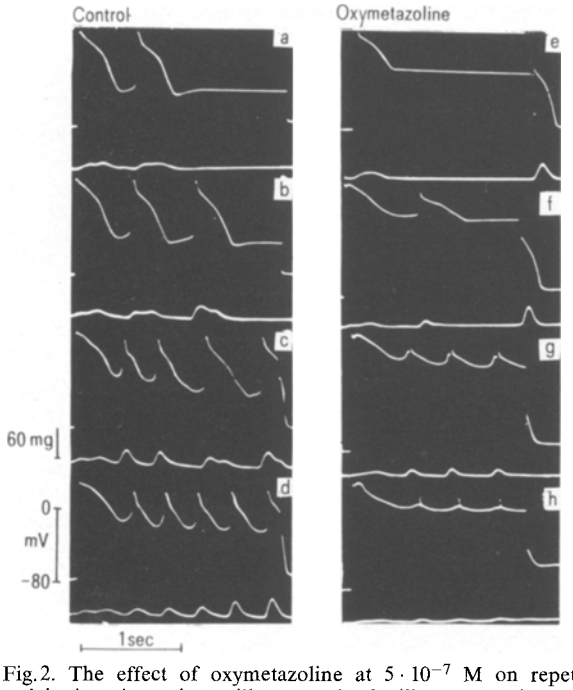

Fig. 2. The effect of oxymetazoline at $5 \cdot 10^{-7}$ M on repetitive activity in guinea-pig papillary muscle. Oscilloscope tracings of the repetitive firings (the upper traces) and isometric contraction (the low traces) are presented. In control series the rate of automaticity became larger with the increase of the depolarizing current intensities. The values of current were: *a, e* $8.6 \cdot 10^{-5}$ A, *b* $12.3 \cdot 10^{-5}$ A, *c* $13.6 \cdot 10^{-5}$ A, *d* $14.8 \cdot 10^{-5}$ A, *f* $9.3 \cdot 10^{-5}$ A, *g* $11 \cdot 10^{-5}$ A, *h* $13.6 \cdot 10^{-5}$ A. In oxymetazoline series (30 min after administration), only oscillatory potentials can be elicited by the larger currents. The ionic nature of the small spikes superimposed on the crests of the membrane oscillations was not resolved in the present work.

Results and discussion. Pharmacological evidence for NR participation in the LVA genesis. Our conclusion is based on the following data. Tyramine ($5 \cdot 10^{-5}$ M–10^{-4} M), known to produce leakage of noradrenaline from presynaptic storage granules[14,15], causes potentiation of LVA (n = 7). Such an effect appears after a latency delay (nearly 15–20 min) which is compatible with the indirect presynaptic action of this compound. Typically tyramine elevates the LVA rate and amplitude (fig. 1 e–h), but diminishes the current threshold of LVA activation.

Potentiation of the associated contraction is also evident in this typical figure. Moreover, tyramine induces automaticity in papillary muscle which cannot display it in control conditions. These changes were completely prevented or successfully antagonized by the β-blocker alprenolol (10^{-6} M–$5 \cdot 10^{-6}$ M). Disappearance of LVA under long-lasting tyramine exposure (1 h) seems to be related to the NR depletion noted under such conditions[14]; the effect of tyramine is consistently reduced or eliminated in reserpinized rabbit and guinea-pig muscles. Thus, all the obtained LVA modulations may be explained by indirect presynaptic action of tyramine.

In contrast to the potentiating action of tyramine, the preferentially presynaptic α-agonist oxymetazoline (n = 5), putative inhibitor of NR from myocardial adrenergic nerve endings[14,15] decreased the LVA rate and amplitude but increased the current threshold of its activity. As a rule, only small oscillations in the diminished voltage range were observed in the oxymetazoline series (fig. 2 e–h) as compared to the control (fig. 2 a–d). The effect of oxymetazoline was readily reversed. No essential changes in LVA were found under atropine administration (10^{-6} M). However, the β-antagonist alprenolol (10^{-6}–10^{-5} M) decreases the parameters of LVA and the associated contractions as illustrated by the representative experiment in fig. 3. Finally, all the results obtained favour the suggestion that NR may be important in LVA genesis in addition to previously revealed factors[1–8].

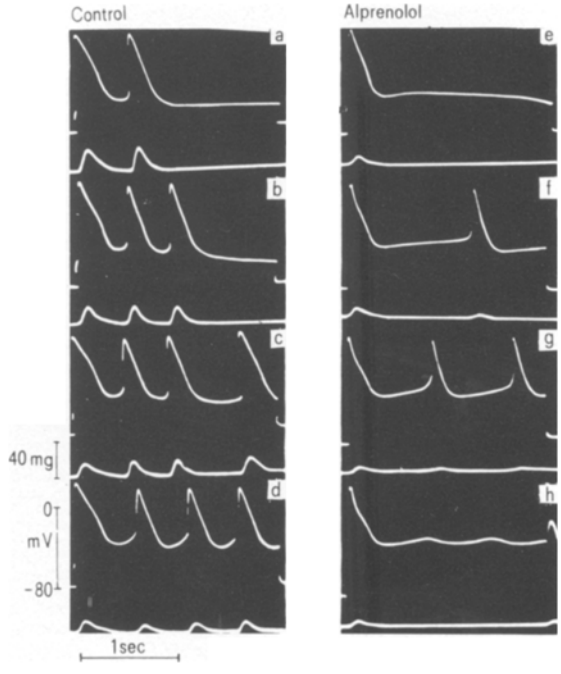

Fig. 3. Alprenolol (10^{-5} M)-reduced repetitive activity (the upper traces) and isometric contractions (the low traces) in guinea-pig papillary muscles; 25 min exposure to β-blocker. Current intensities were: *a, e* $3.8 \cdot 10^{-5}$ A, *b, f* $3.9 \cdot 10^{-5}$ A, *c, g* $4.0 \cdot 10^{-5}$ A, *d, h* $4.2 \cdot 10^{-5}$ A.

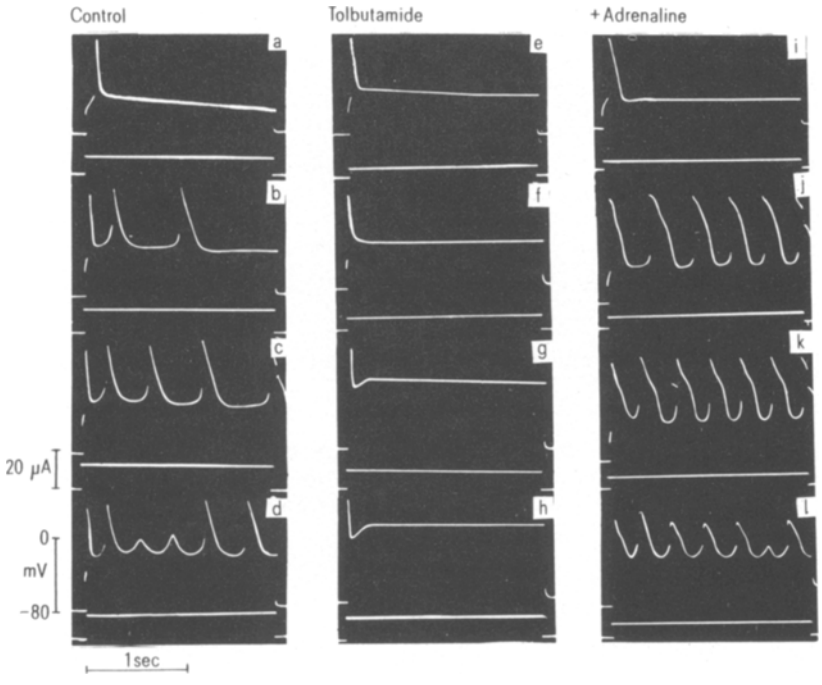

Fig. 4. Elimination of repetitive activity (upper traces) in rabbit papillary muscle by an inhibitor of c-AMP-dependent protein kinase, tolbutamide ($5 \cdot 10^{-5}$ M). The depolarizing current steps are given in the lower traces.

The role of c-AMP-dependent membrane phosphorylation in LVA genesis. There is an increasing amount of evidence which suggests that the functional activity of the calcium channels or of calcium-mediated automaticity is controlled by the intracellular level of c-AMP[16-20]. In addition, we have made some pharmacological tests to obtain deeper insight into the participaction of the c-AMP system in LVA generation; the former is expected to be activated by NR. For instance LVA exhibits a rather high sensitivity to an inhibitor of c-AMP-dependent protein kinase, tolbutamide[21] and an inhibitor of membrane adenylate cyclase, RMI 12330A[22].

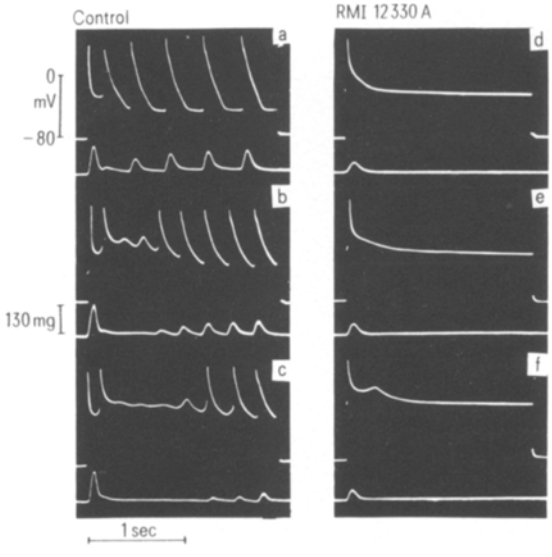

Fig. 5. Supression of repetitive activity and isometric contraction in rabbit papillary muscle by an inhibitor of membrane adenylate cyclase RMI 12330 A, at 10^{-4} M. 30 min after exposure. The current intensities were: *a* $12 \cdot 10^{-5}$ A, *b* $12.6 \cdot 10^{-5}$ A, *c* $13 \cdot 10^{-5}$ A, *d* $12.7 \cdot 10^{-5}$ A, *e* $13.5 \cdot 10^{-5}$ A, *f* $14 \cdot 10^{-5}$ A.

Both inhibitors (n = 5) markedly decreased or even supressed LVA in rabbit papillary muscles. Figures 4B and 5B demonstrate preparations made quiescent by tolbutamide ($5 \cdot 10^{-5}$ M) and RMI 12330A (10^{-4} M), respectively. In contrast, automaticity could be enhanced by phosphodiesterase inhibitors - papaverine (10^{-5} M) or 3-isobutyl-I-methylxanthine (10^{-4} M), and by exogenous adrenaline (10^{-7} M) (the data are not shown). Simultaneously, increased force of contraction was found. This may again indicate that the potentiating effects of NR on LVA generation are mediated by the elevation of intracellular c-AMP. Finally, the present findings are compatible with the hypothesis of potential arrhythmogenicity of hyperactivity of the c-AMP system which is typical for ischaemic myocardium[23,24].

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Polyphosphoinositide metabolism in erythrocytes from spontaneously hypertensive rats

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Summary. Incorporation of ^{32}P into di- and triphosphoinositides of erythrocytes from 1-month-old spontaneously hypertensive rats was lower, and diphosphoinositide content higher, than in controls. During development of hypertension these initial differences decreased and were even reversed.

Polyphosphoinositides (PPI) are involved in the regulation of calcium binding[2] and are supposed to control cell membrane permeability for monovalent cations[3].

An increase in passive permeability for monovalent cations and an alteration of calcium distribution in cell membranes from spontaneously hypertensive rats (SHR) and patients with essential hypertension were found in vascular smooth muscles[4], cardiomyocytes[5], erythrocytes[6] and adipose tissue[7]. Hence membrane alterations are considered as a possible cause for the development of essential hypertension[8].

An increase of PPI content in erythrocyte membranes of 2-month-old SHR was reported[9]. We studied erythrocyte PPI content and incorporation of ^{32}P into erythrocyte PPI throughout the development of hypertension in SHR (1-, 2- and 4-month-old rats).

Materials and methods. We used 1-, 2-, and 4-month-old male spontaneously hypertensive rats (SHR, Kyoto Wistar) and inbred normotensive Wistar rats (NWR) of the same age and sex. Both groups of 1-month-old rats had the same systolic blood pressure (115 mm Hg), but the values of 2- and 4-month-old SHR were significantly higher (160 and 205 mm Hg). Blood pressure was measured by tail plethysmography. $Na_2H^{32}PO_4$ was injected i.p. (2 mCi/kg b.wt).

After 90 min the rats were decapitated and the blood collected from each separately in physiological solution with heparin. Haematocrit was determined. From packed erythrocytes the lipids were extracted[10] and the values of specific activity of inorganic phosphate[11] determined. The lipid extracts were chromatographed on formaldehyde-treated paper[12]. Mono-, di- and triphosphoinositide (MPI, DPI and TPI) were analyzed. We measured the radioactivity of the phospholipid-containing paper strips in dioxan scintillation solution with an LKB Wallac 81,000 liquid scintillation counter. The same paper strips were then used for phosphate determination[13] after mineralization of the paper. The content of phospholipids is given in nmoles/ml of intact erythrocytes, and the ^{32}P incorporation rate is expressed as relative specific activity (RSA), that is, as the ratio of specific activity (cpm/μg phosphorus) of each fraction to the specific activity of erythrocyte inorganic phosphate, multiplied by 100.

Results and discussion. We found alterations of phosphoinositide content of erythrocytes between SHR and NWR in accordance with others[9].

Moreover, the alterations of phosphoinositide content as well as differences of ^{32}P incorporation into phosphoinositides were age-dependent. MPI content of erythrocytes was significantly lower in 2-month-old SHR compared with NWR. Furthermore, ^{32}P incorporation into DPI and TPI was lower in 1-month-old SHR (53% and 65% of control groups) and the DPI-content higher (220% of control group) (see table). During development of hypertension, the initial difference between 1-month-old SHR and NWR fell to nearly zero at 2 months, and was even reversed for 4-month-old rats.

The initial differences at 1 month might be explained by a lower activity of the phosphorylation/dephosphorylation processes for erythrocyte phosphoinositides in SHR. This lower activity is presumably connected with the altered

Content (nmole/ml of erythrocytes) and relative specific activity (RSA) of phosphoinositides in erythrocytes of spontaneously hypertensive (SHR) and normotensive Wistar rats (NWR) depending on age (months)

		1 month	2 months	4 months
		nmole/ml of erythrocytes		
TPI	SHR	78.9±2.5	68.4±5.1	90.5 ±4.8
	NWR	69.8±1.9	58.3±4.5	96.8 ±2.0
	p			
DPI	SHR	57.7±3.2	25.6±2.1	38.9 ±3.2
	NWR	26.3±1.6	32.7±5.2	53.7 ±3.4
	p	<0.001		<0.05
MPI	SHR	335±14	222±5	285±13
	NWR	306±14	243±6	313±15
	p		<0.05	
		RSA		
TPI	SHR	29.9±0.7	24.1±1.0	17.3 ±0.8
	NWR	45.6±1.9	21.4±0.7	12.1 ±0.6
	p	<0.001		<0.001
DPI	SHR	17.8±0.8	19.5±2.2	10.22±0.8
	NWR	31.1±1.4	17.8±2.0	7.48±0.9
	p	<0.001		<0.05
MPI	SHR	2.31±0.12	1.40±0.13	0.95±0.04
	NWR	2.30±0.12	1.63±0.16	0.89±0.05

Means± SEM for 5 animals. The significance of the differences between means of SHR and NWR was assessed by Student's t-test.