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The Influence of the Neuropeptide Galanin on the Contractility and the Effective Refractory Period of Guinea-pig Heart Papillary Muscle Under Normoxic and Hypoxic Conditions

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Abstract

The aim of this study was to discover the effects of galanin, a neuropeptide comprising 29 amino acids capable of activating inward-rectifier K^+ channels (I_{K1}) in cardiomyocytes, on the force of contraction (Fc), velocity of contraction (+dF/dt), velocity of relaxation (−dF/dt) and effective refractory period (ERP) of guinea-pig heart. The influence of galanin on the time-course of hypoxia-induced disturbances in contractility and ERP was also examined. Experiments were performed on the isolated right ventricle papillary muscle of guinea-pig heart.

In the concentration range 0·001–0·01 μM, galanin had no significant effect on the measured parameters. At 0·03 and 0·1 μM, galanin exerted a positive inotropic action and prolonged ERP. Further increasing the concentration led to a negative inotropic action and significant shortening of ERP. Although simulated hypoxia induced a significant drop in Fc, +dF/dt and −dF/dt, and a significant shortening of ERP, recovery of all the measured parameters was complete after 10 min reperfusion. In the presence of 0·03 and 0·1 μM galanin the effect of hypoxia on the contractility of papillary muscle was more profound and reperfusion did not result in complete recovery. In contrast, addition of 1 μM galanin to the hypoxic solution significantly protected the muscle and recovery of the tissues during reperfusion was rapid and complete (in 5 min).

One can conclude that galanin at lower concentrations induced a positive inotropic action and a prolongation of ERP, but increased the sensitivity of heart muscle to hypoxia. At higher concentrations however, galanin exerted a negative inotropic action but protected the muscle against hypoxia-induced disturbances in contractility.

Galanin is a 29-amino-acid neuropeptide with multiple effects in the central and peripheral nervous system (Bedecs et al 1995). It has been shown in man that galanin depresses the release of noradrenaline (degli Uberti et al 1995) and there is evidence for the presence of this neuropeptide in rat, mouse, guinea-pig, rabbit, cat and dog heart (Xu et al 1995). Although a galanin receptor (hGalR1) has been identified in heart muscle in man (Sullivan et al 1997), the function and role of galanin in the human heart is not known. Experiments performed in mudpuppy cardiac tissue have demonstrated that galanin activates an inwardly-rectifying potassium current (Parsons & Merriam 1993). Other mechanisms of action of galanin, e.g. stimulation of ATP-sensitive K^+ channels (de Weille et al 1988), inhibition of voltage-gated Ca^{2+} channels (Homaidan et al 1991) or inhibition of adenylyl cyclase (Amiranoff et al 1989) have not been shown in heart muscle.

The aim of the current study was to investigate the effects of galanin on the contractility and effective refractory period (ERP) of guinea-pig heart papillary muscle, under normoxic and hypoxic conditions to discover the relationship between inotropic effects and the influence of galanin on the time course of the contractility and ERP of the myocardium during hypoxia.

Materials and Methods

Animals

Experiments were performed on guinea-pigs of either gender, 350–450 g. The animals were housed

in wire-mesh-bottom cages and kept under standard laboratory conditions (12-h light–dark cycle, 21–24°C, humidity 50–55%), with food (Murigran chow pellets, Bacutil, Motycz, Poland) and tap water freely available.

Experimental procedures

Guinea-pigs were anaesthetized by intraperitoneal administration of pentobarbital (60 mg kg^{-1}), the thorax was opened, and the heart was quickly removed and placed in the preparation dish with modified, ice-cold Krebs–Henseleit solution, aerated with carbogen, where the right ventricle papillary muscles were prepared. After preparation, papillary muscle (length > 3 mm, dia. < 1 mm) was mounted in a 2-mL organ bath (Steiert Organ bath, type 813 with DC temperature controller type 319; HSE, Germany) and attached to an isometric force-transducer (F-30, HSE). Isolated tissue was superfused with Krebs–Henseleit solution containing (mM): NaCl 119; $CaCl_2$ 2·5; KCl 4·8; $MgSO_4$ 1·2; KH_2PO_4 1·2; $NaHCO_3$ 24·8 and glucose 11·5. The rate of perfusion was approximately 7 mL min^{-1} (peristaltic pump, type 371; Unipan, Poland). The solution was aerated with 95% O_2–5% CO_2, at 37 ± 0·5°C. Resting tension, carefully adjusted to obtain the maximum force of contraction, was 0·4 ± 0·25 mN (n = 12). Tissues were electrically paced by two silver electrodes in contact with the muscles, with square waves, 1 Hz, 3 ms duration, threshold voltage +20%, generated by an electronic stimulator (ST-02; Experimetria, Hungary). The developed tension (Fc) and the velocity of contraction (+dF/dt) and relaxation (−dF/dt) were measured by means of an F-30 isometric force–displacement transducer and bridge amplifier with a type 336 differentiator (HSE). The signals were displayed on a digital storage oscilloscope (VC-6525; Hitachi, Japan) and a personal computer (PC 486) running HIMES software (Hitachi, Japan) enabled measurement of effective refractory period (ERP) as the shortest interval between two stimuli (S_1–S_2) inducing an increase in force amplitude of at least 25% (Kocic 1996). All muscles were equilibrated for 60 min in oxygenated Krebs–Henseleit solution. Simulated hypoxia was achieved by superfusion of the tissue with modified nutrient solution (NaCl 136·2; $CaCl_2$ 2·2; KCl 6·5; $MgSO_4$ 1·2; KH_2PO_4 1·2; $NaHCO_3$ 10 and glucose 11·5 mM gassed with N_2 95%–CO_2 5%, pH 6·8, at 32°C, for 25 min; Németh & Papp 1991). Reperfusion was achieved by switching from hypoxic solution to oxygenated Krebs–Henseleit solution. Measurement of Fc, +dF/dt, −dF/dt, and ERP was performed, after a period of equilibration, after 5, 10, 20 and 25 min of hypoxia.

Drugs

Galanin was synthesized and generously donated by Dr P. Rekowski and co-workers from the Faculty of Chemistry, University of Gdansk, Poland. The drug was dissolved in distilled water. Stock solution, 1 mL (1000 μM) was obtained by mixing 0·1 mL distilled water with 0·9 mL Krebs–Henseleit solution. The amount of distilled water in the perfusion solution was < 0·05% and had no effect on the baseline parameters of contractility.

Statistics

Data are expressed as means ± s.e.m. Differences between control values and means after different periods of hypoxia and reperfusion were evaluated by two-way analysis of variance followed by a Newman–Keuls test or two-tailed Student *t*-test, data being grouped as appropriate. The computer program used was Pharmacological Calculation System Pharm/PCS, version 4 which is based on the Manual of Pharmacological Calculations with Computer Programs (Tallarida & Murray 1987). $P < 0·05$ was considered to be indicative of statistical significance.

Results

Results, expressed as the percentage of the control values, which were: for Fc, 0·7 ± 0·3 mN mm^{-2}, n = 20 (1 mN = 0·1 g); for +dF/dt, 3·9 ± 1·7 mN s^{-1} mm^{-2}, and for −dF/dt, 2·1 ± 0·9 mN s^{-1} mm^{-2}.

Effects of galanin on the baseline contractility and effective refractory period of isolated guinea-pig heart papillary muscle

Figure 1 shows the effects of galanin on the force of contraction (Fc), velocity of contraction

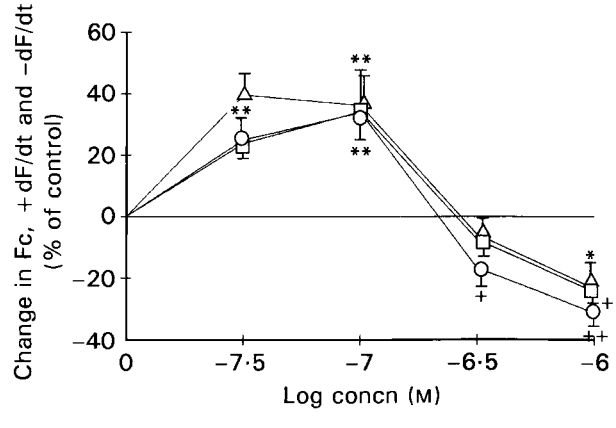

Figure 1. The effects of different concentrations of galanin on the force of contraction (Fc, ○), the velocity of contraction (+dF/dt, □) and the velocity of relaxation (−dF/dt,) of guinea-pig isolated papillary muscle. Data are means ± s.e.m. of results from five experiments. $^{**}P < 0·01$, significantly different from baseline values; $+P < 0·05$, $++P < 0·01$, significantly different from the maximum positive inotropic action of galanin (two-way analysis of variance and Newman–Keuls test).

Table 1. The effects of different concentrations of galanin on the effective refractory period of guinea-pig papillary muscle.

Log concn galanin (M)	Effective refractory period (ms)
0	97·5 ± 7
−7·5	116 ± 7*
−7	120 ± 7*
−6·5	68 ± 16†
−6	64 ± 23‡

Data are means ± s.e.m. of results from five experiments. *$P < 0.01$; significantly different from baseline values. †$P < 0.05$, ‡$P < 0.01$, significantly different from the maximum prolongation of effective refractory period induced by galanin (two-way analysis of variance and Newman–Keuls test).

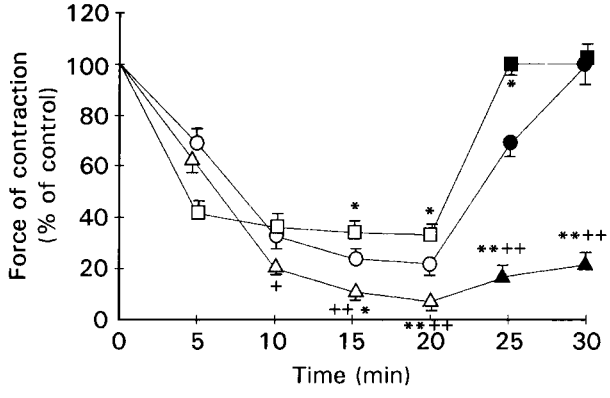

Figure 2. Time-course of the force of contraction (Fc) in guinea-pig isolated papillary muscle during 25 min hypoxia and 10 min reperfusion in the presence of 0·03 and 1 μM galanin. ○, ● Fc during ischaemia and reperfusion, respectively, in the absence of galanin; □, ■ +dF/dt during ischaemia and reperfusion, respectively, in presence of 1 μM galanin; △, ▲ −dF/dt during ischaemia and reperfusion, respectively, in the presence of 0·03 μM galanin. Data are means ± s.e.m. of results from five experiments. *$P < 0.05$; **$P < 0.01$, significantly different from control values obtained in the absence of galanin; +$P < 0.05$, ++$P < 0.01$, significantly different from the values obtained in the presence of 1 μM galanin (two-tailed Student t-test, grouped data).

(+dF/dt) and velocity of relaxation (−dF/dt). The curves show the action of the drug to be biphasic, with a positive inotropic action at lower concentrations and negative action at higher concentrations. Table 1 depicts the effect of different concentrations of galanin on the duration of the effective refractory period (ERP). Although low concentrations of galanin prolong the duration of ERP, higher concentrations shorten it significantly.

The influence of galanin on the time-course of contractility and effective refractory period of guinea-pig papillary muscle during simulated hypoxia and reperfusion

Figure 2 and Tables 2 and 3 show the influence of 0·03 and 1 μM galanin on changes in the force of contraction, velocity of contraction, velocity of relaxation and ERP of isolated guinea-pig papillary muscle during hypoxia and reperfusion. It is noteworthy that simulated ischaemia caused a significant drop in Fc, +dF/dt and −dF/dt and shortening of ERP, but all the parameters recovered completely after 10 min reperfusion. During hypoxia the decrease in all the parameters was faster and stronger in the presence of 0·03 μM galanin and there was no recovery of the measured parameters during 20 min reperfusion. In the presence of 1 μM galanin, after 25 min hypoxia Fc, +dF/dt and −dF/dt were significantly higher than for the control group and complete recovery of all the parameters was observed after 5 min reperfusion. Although addition of 0·03 μM galanin induced a non-significant reduction in the duration of the ERP compared with the control value, after 25 min hypoxia this value was significantly shorter than the value obtained in the presence of 1 μM galanin (Table 3).

Discussion

The main findings of this study are that galanin (0·03, 0·1, 0·3 and 1 μM) exerted a biphasic action on the contractility and effective refractory period of guinea-pig isolated papillary muscle. At the lowest concentrations (0·03 and 0·1 μM) a positive inotropic action was observed, but at the highest concentrations (0·3 and 1 μM) galanin had a negative inotropic action. It has also been shown that in

Table 2. The velocity of contraction and relaxation of isolated guinea-pig papillary muscle after 25 min simulated hypoxia and after 20 min reperfusion in the absence of galanin (control group) and in the presence of 0·03 and 1 μM galanin.

	Velocity of contraction		Velocity of relaxation	
	After hypoxia	After reperfusion	After hypoxia	After reperfusion
Control	24 ± 4	99 ± 7	21 ± 3	98 ± 8
Galanin 0·03 μM	10 ± 3*†	17 ± 5	5 ± 2*†	16 ± 2
Galanin 1·0 μM	38 ± 4*	101 ± 6	37 ± 3*	102 ± 5

Data (% of control) are means ± s.e.m. of results from five experiments. *$P < 0.01$, significantly different from control values; †$P < 0.01$, significantly different from values obtained in the presence of 1 μM galanin (two-tailed Student t-test, grouped data).

Table 3. The effective refractory periods of isolated guinea-pig papillary muscles after 25 min simulated hypoxia and after 20 min reperfusion in the absence of galanin (control group) and in the presence of 0·03 and 1 μM galanin.

	After hypoxia	After reperfusion
Control	60±9	99±14
Galanin 0·03 μM	39±10	60±7
Galanin 1·0 μM	73±5*	101±8

Data (ms) are means±s.e.m. of results from five experiments. *$P < 0.01$, significantly different from the value obtained in the presence of 0·03 μM galanin (two-tailed Student t-test, grouped data).

the presence of 0·03 μM galanin simulated hypoxia induced significantly stronger damage of contractility compared with control values whereas in the presence of 1 μM galanin, papillary muscle was significantly protected against hypoxia-induced decrease in contractility and its recovery was faster than for the control group and was complete. The main points requiring explanation are the mechanisms involved in the inotropic effects of galanin. There is some evidence that galanin activates inwardly-rectifying K^+ conductance in the cardiomyocytes (Parsons & Merriam 1993). None of the other possible mechanisms involved in galanin action—as an inhibitor of voltage-gated Ca^{2+} channels (Kalkbrenner et al 1995), as an inhibitor of adenylyl cyclase (Amiranoff et al 1989) and as an activator of ATP-sensitive K^+ channels (Dunne et al 1989)) were demonstrated in heart muscle, but the significant negative inotropic action of 1 μM galanin suggested either that the activation of $I_{K(ATP)}$ currents or inhibition of Ca^{2+} channels is responsible for this effect. The positive inotropic action of 0·03 and 0·1 μM galanin is more likely to be a result of activation of inwardly rectifying K^+ channels, the only mechanism of action of galanin of those mentioned above that can lead to depolarization of the cells. The results presented here also indicate that pretreatment with the lowest concentrations of galanin aggravates the sensitivity of guinea-pig isolated papillary muscle to hypoxia, but pretreatment with the highest concentrations protects it against hypoxia-induced disturbances in contractility. The implication of these data is that the action of galanin in guinea-pig isolated papillary muscle is concentration-dependent. At low concentrations galanin induces a positive inotropic action and increases the sensitivity of heart muscle to hypoxia, probably because of the activation of the inwardly-rectifying K^+ channels, and a positive inotropic action enhances the oxygen demand of the myocardium, which might be the reason for the low resistance to hypoxia under such conditions. The negative inotropic action and protection of heart muscle against hypoxia is probably a result of the activation of ATP-sensitive K^+ channels.

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