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Influence of chronic treatment of rats with isoprenaline and calcium channel blockers on response of isolated right ventricle to noradrenaline

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Influence of chronic treatment of rats with and calcium channel blockers (CCBs) and isoprenaline (ISP) on responses to noradrenaline (NA) was investigated on electrically - driven isolated right ventricle preparations. The ventricles were obtained from animals treated with chronic ISP or CCBs alone and chronic nifedipine, verapamil, diltiazem or nimodipine plus chronic ISP. A decreased response to NA as evidenced by an increase in EC_{50} for contraction which was observed in chronic ISP- treated preparations may be due to development of desensitisation (down-regulation) of beta-adrenoceptors. In chronic CCB-treated preparations there was a significant decrease in the EC_{50} of NA and decreased contractile response suggesting an increase in the beta-adrenoceptors and decreased availability of calcium, respectively. In chronic CCBs + ISP treated preparations further decreases in the EC_{50} values were observed suggesting that the voltage gated L-type Ca^{2+} channels may be affected directly or indirectly by change in beta-adrenoceptor activity. By the above results a proposed mechanism of interrelationship of beta-adrenoceptors with voltage gated L - type calcium channels in cardiac muscle is supported.

Prolonged exposure of intact tissues to high agonist concentrations produces receptor desensitization[1]. The effect of beta - adrenoceptor stimulation on the heart is in part mediated by activation of L-type calcium channels in the cardiac muscle cells. Exposure to isoprenaline (ISP) results in down-regulation of L-type Ca^{2+} channels in cultured myocytes from chick heart[2]. However L-type Ca^{2+} channel down-regulation appears not to accompany agonist mediated beta-adrenoceptors down-regulation in hearts of Wistar rat pups (2-3 days old)[3]. Using the right ventricle preparation as the experimental model attempt has been made to study the interaction of isoprenaline and calcium channel blockers on noradrenaline (NA) induced changes in contractile activity of this tissue.

Preparation of rat isolated ventricle for recording of contractions—Male Wistar rats (250-350 g) were sacrificed by a sharp blow on the head and cutting the neck blood vessels. The heart was rapidly removed and placed in a Petridish containing oxygenated pre-warmed (35°C) Ringer - Locke physiological salt solution of the following composition (m*M*) : NaCl, 15.4; KCl, 5.6; $CaCl_2$, 2.2; $NaHCO_3$, 6 and glucose - 11.1. The solution always contained guanethidine (1×10^{-6} *M*) and atropine (1×10^{-6} *M*). The free wall of the right ventricle was excised and the intact right ventricle was suspended[4] in a 30 ml organ bath containing Ringer - Locke physiological salt solution (35°±0.5°C, bubbled with oxygen, 1 g tension). After equilibration for 45 min, the ventricle was electrically driven by square wave pulses (5 PPS, 5ms with 1ms delay at 50V supramaximal voltage. The electrodes were attached to a research model SS-48 stimulator (Recorders and Medicare System, Chandigarh).

Concentration-response curves of NA were obtained non-cumulatively on a 15 min cycle. The maximal increase in contractile force of ventricle for each concentration of NA was measured.

Chronic drug treatment schedules—Groups of 6-10 rats received chronic treatment with drugs as follows:

(a) ISP as Isoprenaline sulphate dissolved in 0.9% saline was administered in a dose of 100 µg/kg, ip once daily for 7 days.

(b)Verapamil (30 mg/kg) and diltiazem (20 mg/kg) dissolved in triple glass-distilled water and nifedipine (10 mg/kg) and nimodipine (20 mg/kg) dissolved in the solvent (polyethylene glycol 400-969 g, glycerin 60 g, water 100 g) were administered orally via a Ryles tube once daily for 28 days. During the last 7 days, some rats were also given ISP, [100 µg/kg, ip daily as mentioned in (a) above].

Drugs—Atropine sulphate and guanethedine (1-(2-guanidinoethy)octa hydroazocine) were obtained from Sigma Chem. Co. The following drugs were received

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as free gifts : isoprenaline sulphate (Burroughs Wellcome (India) Ltd; Mumbai); diltiazem (Sun Pharmaceutical Industries; Baroda); verapamil and nifedipine (Torrent Pharmaceutical Ltd; Ahmedabad); nimodipine (U.S. Vitamins; Mumbai). Polyethylene glycol 400 (E. Merck (India) Ltd.; Mumbai) and glycerin I.P. (Metro Pharmaceutical Industry, Wadhwan City) were used as solvents.

Statistical methods—Only one agonist was used for getting concentration-response curve in a given preparation. Contractile force was expressed as gram of tension. The EC_{50} values were determined by linear interpolation from each concentration-response curve[5]. The results were expressed as mean ± SEM and analyzed by one-way classification analysis of variance "Newman-Keuls test"[6]. In all statistical analysis values of $P < 0.05$ were considered as significant.

Results are presented in Table 1.

Direct stimulation of the right ventricle (5 PPS, 5 ms, 1 ms, 50 v) contracted the muscle. NA (3.51×10^{7} M–1.40×10^{-6} M) increased the force of contraction in a concentration-related manner. Significant differences were noted when the control EC_{50} value of NA and its maximal response were compared with the corresponding values obtained in ISP - treated preparations.

There was a significant difference in the EC_{50} values of NA and its maximal contractile response when compared with the respective values obtained from the ventricles of animals treated with chronic verapamil, nifedipine, nimodipine or diltiazem or chronic verapamil, nifedipine or nimodipine + ISP treated preparations. Chronic verapamil, nifedipine, nimodipine or diltiazem + ISP treated rats showed no significant difference between their maximal contractile force when compared to the responses of the ventricles obtained from animals treated alone with chronic ISP or chronic verapamil or nifedipine or nimodipine. However, a significant difference in the EC_{50} values was noted among the similar values from these groups of animals. Comparison of the EC_{50} values of chronic diltiazem- treated rats and chronic diltiazem + ISP treated rats showed no significant difference.

Hausdorff *et al.*[7], suggested that long-term application of agonists leads to a desensitization and finally down-regulation of beta-adrenoceptors. In the present study a decreased response to NA observed in the chronic ISP-treated preparations as compared to NA response in drug-native control animals may be due to rapid desensitization (down-regulation) of beta-adrenoceptors which appears to be a most likely consequence of the uncoupling of receptor from the G_S protein[1] as a result of chronic exposure of receptors to a selective beta-adrenoceptor agonist.

In chronic CCBs-treated preparations a significant decrease in the EC_{50} values of NA as compared to similar values obtained in control and chronic ISP-treated preparations suggests an increase in the sensitivity of beta-adrenoceptors. However, chronic treatment with Ca^{2+}-channel blockers also caused a decrease in the maximal contractile response which suggests a decreased liability of calcium due to blockade of L-type Ca^{2+}-channel in ventricle muscle[8].

With chronic CCBs+ISP treatment (except diltiazem), there was a significant decrease in the EC_{50} values suggesting that voltage gated L-type Ca^{2+} channels could be affected by binding directly to G-pathway. The suggestion regarding binding to G - pathway is in line with the proposal of Hartzell and Fischmeister[9] for regulation of voltage gated L-type Ca^{2+} channel in cardiac muscle. According to this model when ISP binds to the beta-adrenoceptors a nucleotide exchange on the α_S, subunit of guanosine stimulating protein (G_S) is accelerated resulting in activated guanosine stimulated alpha protein ($G_S\alpha$). $G_S\alpha$ can then activate the calcium channels directly by binding to direct G-protein pathway or indirectly to phosphorylation pathway leading to activation of adenylcyclase which synthesizes cAMP and that in turn activates cAMP dependent protein kinase thought to phosphorylate the Ca^{2+} channels. cAMP may be degraded by the variety of phosphodiesterases and the Ca^{2+} channel may be dephosphorylated by one or several phosphatases. Radio ligand binding studies will be required to confirm the proposed mechanism .

However, the non-significant changes observed after chronic diltiazem + ISP treatment would suggest that this effect may be related to the occurrence of different isoforms of L-type calcium channels[10] or due to insignificant role of the autonomic mechanisms in the net haemodynamic effect of diltiazem as compared to other CCBs[11].

Non-significant change in the maximal contractile force among various comparison groups (i.e. chronic ISP- or chronic CCBs-treated preparations versus chronic CCBs+ISP) would suggest decreased availability of calcium[12].

Table 1—Mean EC_{50} values and mean maximal contractile force elicited with NA in ventricles of control rats and rats treated with chronic ISP, chronic verapamil + ISP, chronic nifedipine + ISP, chronic nimopidine + ISP and chronic diltiazem + ISP(n = 5 - 6 for each group of observation)

Variables	Drug-naive	Chronic ISP	Chronic verapamil	Chronic verapamil + ISP	Chronic nifedipine	Chronic nifedipine + ISP	Chronic nimodipine	Chronic nimodipine + ISP	Chronic diltiazem	Chronic diltiazem + ISP
EC_{50} (M)	4.677×10^{-7}	7.022×10^{-7}	3.963×10^{-7} (S.E.± 0.093;	2.188×10^{-7} D 0.395)	2.421×10^{-7} (S.E.± 0.075;	1.282×10^{-7} D 0.321)	1.484×10^{-7} (S.E.± 0.025;	8.872×10^{-7} D 0.107)	1.294×10^{-7} (S.E.± 0.027;	1.318×10^{-7} D 0.116)
Maximal Contractile force (g)	4.07	2.09	2.37 (S.E.± 0.218;	2.28 D 0.928)	2.02 (S.E.± 0.253;	2.14 D 1.084)	2.95 (S.E.± 0.264;	2.89 D 1.055)	2.70 (S.E.± 0.256;	2.70 D 1.096)

S.E. : standard error of mean of the corresponding groups; D : difference between any two means required for 5% significance
The results were analyzed by one way classification of analysis of variance "Newman - Keul test". Values of $P < 0.05$ were considered significant and are represented by the asterisk sign*.

Comparison groups	EC_{50} (M)	Maximal contractile force (g)
(a) Drug naVve vs chronic ISP	*	*
(b) Drug naVve vs chronic verapamil	*	*
(c) Drug naVve vs chronic verapamil + chronic ISP	*	*
(d) Chronic ISP vs chronic verapamil + chronic ISP	*	NS
(e) Chronic verapamil vs chronic verapamil + chronic ISP	*	NS
(f) Drug naVve vs chronic nifedipine	*	*
(g) Drug naVve vs chronic nifedipine + chronic ISP	*	*
(h) Chronic ISP vs chronic nifedipine + chronic ISP	*	NS
(i) Drug naVve vs chronic nifedipine + chronic ISP	*	NS
(j) Drug naVve vs chronic nimodipine	*	*
(k) Drug naVve vs chronic nimodipine + chronic ISP	*	*
(l) Chronic ISP vs chronic nimodipine + chronic ISP	*	NS
(m) Chronic nimodipine vs chronic nimodipine + chronic ISP	*	NS
(n) Drug naVve vs chronic diltiazem	*	*
(o) Drug naVve vs chronic diltiazem + chronic ISP	*	*
(p) Chronic ISP vs chronic diltiazem + chronic ISP	*	NS
(q) Chronic diltiazem vs chronic diltiazem + chronic ISP	*	NS

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