Indian J Physiol Pharmacol 1999; 43 (3) : 389–392

EVALUATION OF THE ACTIVITY OF RECEPTOR-OPERATED Ca^{2+} CHANNELS IN RAT PORTAL VEIN IN INDUCED HYPERTHYROIDISM

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(Received on March 25, 1999)

Abstract : The activity of receptor-operated Ca^{2+} channels (ROCCs) was studied in rat portal vein in L-thyroxine-induced experimental hyperthyroidism. The following parameters were evaluated : 1. NE-stimulated ^{45}Ca influx. 2. $CaCl_2$–induced contractile responses in Ca^{2+} free NE-stimulated tissues to calculate EC_{50} value of $CaCl_2$. The NE (10^{-6}mol) stimulated ^{45}Ca influx and the mean EC_{50} value of $CaCl_2$ did not differ significantly in portal veins isolated from hyperthyroid rats as compared to those of euthyroid control rats. The study revealed no significant change in the functional status of ROCCs in experimental hyperthyroidism.

Key words : hyperthyroidism rat portal vein ROCCs
^{45}Ca influx $CaCl_2$ contractions Norepinephrine

INTRODUCTION

Calcium ions (Ca^{2+}) play a pivotal role in the excitation-contraction coupling process of myocardium and smooth muscles of different cellular systems (1). Calcium ions either enter the cells through membrane channels or are released from the stores via ROCCs when the receptor-effector system is stimulated. The operation of these Ca^{2+} channels is subject to regulatory influences which may be of pathological, physiological or pharmacological in nature (2). For example norepinephrine induced actions on vascular smooth muscle are mediated by activation of alpha adrenoceptor-operated Ca^{2+} channels (3).

In the vascular smooth muscle, the effects of thyroid hormones on adrenoceptors are controversial (4-7). Therefore, the present investigation was undertaken to determine the influence of hyperthyroidism on the activity of alpha-adrenoceptor coupled ROCCs in isolated rat hepatic portal veins.

METHODS

The studies were conducted on adult, healthy, male albino rats (150–180 g), of sprague-Dawley strain, produced from the Laboratory Animal Resource Section of I.V.R.I., Izatnager (U.P.). Rats were maintained in polypropylene cages under

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standard laboratory conditions. Following acclimatization period of one week in the laboratory, rats were divided randomly into 2 groups of 10 animals each. First group was kept as alkaline saline control and in the second group hyperthyroidism was induced by L-thyroxine (Sigma, USA) dissolved in alkaline saline solution, (0.001 N Na OH in 0.9% NaCl solution) injected subcutaneously (0.75 mg/kg), once daily for 7 days (8). A high T3 and T4 hormone status was confirmed by RIA using T3 and T4 kits (BRIT, India). The hyperthyroid rats and alkaline saline-treated euthyroid control rats were utilized 24 h after the last injection for isolating the intact hepatic portal veins.

To study the $CaCl_2$-induced contractile responses, the intact hepatic portal veins were isolated from both eu-(n=6) and hyper-thyroid (n=6) rats, and cleaned gently. They were then mounted in an organ bath of 20 ml capacity, containing Ca^{2+} free Kreb's solution with EGTA (EGTA 0.1, NaCl 120, KCl 5.9, $NaHCO_3$ 8.0, $MgCl_2$ 1.2, NaH_2PO_4 1.2 and glucose 11.5 mM). The bath was continuously aerated with atmospheric air and maintained at 37±0.5°C for 15 min. The tissue was then exposed to Ca^{2+} free Kreb's solution without EGTA and left for 60 min with repeated washes at 15 min intervals and at a resting tension of 0.5 g. Subsequently, 10^{-6} mol norepinephrine (NE) (Arterenol, Sigma, U.S.A.) was added to the organ bath (10^{-6} mol). After incubating the tissue for 3 min in the presence of NE, concentration related contractile responses were elicited by adding $CaCl_2$ ($10^{-4} - 3\times10^{2}$ mol) cumulatively. This procedure eliminates participation of voltage-dependent Ca^{2+} channels (VDCCs). The contractions were recorded by means of force displacement transducer (T 305, Ft 1047) connected to a multichannel recorder (Polyrite; Medicare, India), calibrated to record change in the tension generated on g vs mm displacement basis. The EC_{50} values of $CaCl_2$ were calculated by regression analysis using the least squares method (9) and Student "t" test was applied to test the significance.

For quantitation of cellular influx of ^{45}Ca the methods of Godfraind (10) and Batra et al. (11) were employed with modifications. Briefly, after removing all visible connetive tissue and fat from portal veins isolated from eu- and hyper-thyroid rats (n=4 each), the portal veins were equilibrated for 60 min in Tris-buffered saline solution at 37±0.5°C with continuous aeration. After this pre-incubation, the strips were stimulated by NE (10^{-6} mol) for 2 min and incubated for 10 min in ^{45}Ca (BARC, India) containing Tris-buffered saline solution (2 μ Ci/ml). Tissues were then quickly taken out and soaked for 20 min in chilled Ca^{2+} free EGTA containing saline solution with continuous aeration. After washing, the tissues were blotted on a filter paper, placed in scintillation vials and weighed. Samples in scintillation vials were digested by wet oxidation mothod by adding 0.1 ml of concentrated HNO_3 to each vial and heating for 5–10 min in a water bath maintained at 70°C. After cooling to room temperature, 0.1 ml of Tris solution (0.75 mol) was added to each vial followed 4 Bray's scintillation fluid. The readioactivity of the samples was measured in liquid scintillation spectrophotometer (LKB, Wallac 1219, Rack beta scintillation counter). The results of each determination were converted to

'apparent tissue content of ^{45} Ca' using the following formula and Student "t" test was applied to test the significance.

^{45}Ca uptake (m mol/kg wet weight of the tissue) =

$$\frac{\text{CPM in sample}}{\text{Sample weight in kg}} \times \frac{\text{m mol of calcium / L medium}}{\text{CPM / L medium}}$$

(CPM = Counts per minute)

RESULTS

The EC_{50} value of $CaCl_2$ in inducing contractile responses in calcium depleted, NE stimulated, tissues was $5.80\pm0.37 \times 10^{-4}$ mol in euthyroid tissues and $5.75\pm0.89 \times 10^{-4}$ mol in hyperthyroid tissues. These were not significantly different. The threshold concentration of $CaCl_2$ (10^{-4}mol) also did not change (Table I). Similarly, there was no significant change in 2 min NE-stimulated ^{45}Ca influx in the tissue isolated from hyperthyroid rats (0.63±0.10 m mol/kg wet weight of the tissue) as compared to those of euthyroid control rats (0.49±0.12 m mol/kg wet weight of the tissue).

DISCUSSION

The ^{45}Ca influx study was planned to get direct indication of functional alterations, if any, in hyperthyroidism. The NE-stimulated entry of Ca^{2+} into the cells is known to occur through membrane receptor-operated Ca^{2+} channels (ROCCs) that are activated by receptor-agonist interactions with no change in the membrane potential (3). No significant change in ^{45}Ca influx in hyperthyroid tissues as compared to euthyroid ones indicates that the activity of adrenoceptor-operated Ca^{2+} channels (ROCCs) is unaltered. The number of Ca^{2+} channels is known to be associated with the density of receptors specific to a particular agonist. However, no consensus has been reached regarding the density of alpha-adrenoceptors in hyperthyrodism. Previous reports on vascular smooth muscle inidicate that the density of alpha-adrenoceptors in hyperthyroidism is either unchanged (4) or decreased (6, 7). The present findings on ^{45}Ca influx indicate unaltered nature and number of adrenoceptors and therefore, the activity of ROCCs remained unchanged with similar

TABLE I : $CaCl_2$-induced per cent contractile responses in Ca^{2+} free NE-stimulated rat hepatic portal vein preparations.

Group	*$CaCl_2$ concentration (mol)*					*EC_{50} (mol)*
	10^{-4}	*3×10^{-4}*	*10^{-3}*	*3×10^{-3}*	*10^{-2}*	
Euthyroid tissues (n=6)	7 ± 1.7	36 ± 2.9	70 ± 1.7	86 ± 1.6	100	$5.80\pm0.37\times10^{-4}$
Hyper-thyroid tissues (n=6)	12 ± 1.7	40 ± 2.9	66 ± 3.6	84 ± 3.9	100	$5.75\pm0.89\times10^{-4}$

Values are experessed as mean ± S.E.

influx in both eu- and hyper-thyroid states. These results have been further substantiated by the unaltered EC_{50} values of $CaCl_2$ in inducing contractions in NE-stimulated tissues of both the groups. The ROCCs that are activated by NE allow the entry of Ca^{2+} into the cells and the unaltered EC_{50} of $CaCl_2$ might be due to the unchanged number or nature of adrenoceptors and hence the function of ROCCs. These results are in agreement with the report of Fox et al. (4).

Therefore, the present investigation on the receptor-operated Ca^{2+} channels in induced hyperthyroidism indicate that the alpha-adrenoceptor agonist NE did not induce changes on ^{45}Ca influx and $CaCl_2$-induced contractile responses in rat hepatic portal vein which implies that the functional activity of ROCCs in vasculature remain unchanged under high thyroid hormone status.

ACKNOWLEDGMENTS

The authors are thankful to the Director, I.V.R.I., Izatnagar (U.P.), for providing necessary financial and technical facilities to carry out the work.

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