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Modulation of vascular K_{ATP} channels in hypothyroidism

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Abstract

The role of vascular K_{ATP} channels in hypothyroidism-induced decrease in myogenic activity of rat portal vein was examined by using pharmacologically relevant concentrations of K^+ channel ligands. As compared to controls, a significant decrease in the myogenic tone and noradrenaline (10^{-9}–10^{-5} M)-induced contractions was observed in portal veins from hypothyroid rats. In both euthyroid and hypothyroid states, pinacidil (10^{-9}–10^{-5} M) and cromakalim (10^{-9}–10^{-5} M) caused concentration-related inhibition of the myogenic tone (frequency and amplitude). However, hypothyroidism caused a leftward shift in the concentration-response curves of the K^+ channel openers with a corresponding decrease in their IC_{50} values both in the absence and presence of the K_{ATP} channel blocker, glibenclamide (10^{-7} M). Further, concentration-dependent increase in the frequency of myogenic tone by glibenclamide (10^{-8}–3×10^{-6} M) was greater in tissues from hypothyroid rats ($EC_{50} = 2.07 \times 10^{-7}$ M; 95% CL, 1.06–4.05×10^{-7} M) in comparison to controls ($EC_{50} = 8.07 \times 10^{-7}$ M; 95% CL, 0.53–1.22×10^{-6} M). These results suggest that a decrease in the myogenic tone of rat portal vein may possibly be related to an enhanced opening of the K_{ATP} channels in hypothyroidism.

Keywords: Hypothyroidism; K_{ATP} channel; Portal vein; Pinacidil; Cromakalim; Glibenclamide

1. Introduction

The cardiovascular manifestations of hypothyroidism are characterised by low cardiac index, decreased stroke volume and increased systemic vascular resistance (Klein, 1990). Arterial hypotension is another characteristic feature of hypothyroidism (Bradley et al., 1974). Several studies have shown that hypothyroidism causes a decrease in the sensitivity of both conduit and resistance vessels to a variety of vasoconstrictor agents (Scivoletto et al., 1986; Gunasekera and Kuriyama, 1990; Sabio et al., 1994). Similarly, a reduction in myogenic tone was reported to occur in spontaneously contracting portal veins obtained from hypothyroid rats (Chin and Pennefather, 1992). However, the cellular mechanisms, particularly involving membrane ion channels in altering vascular responsiveness in hypothyroid states are not clearly understood.

ATP-sensitive K^+ (K_{ATP}) channels play a key role in determining membrane excitability, resting membrane potential and tone of vascular smooth muscles (Quayle and Standen, 1994). Activation of these channels in reponse to a change in metabolic state gives rise to vasodilator responses as a result of hyperpolarisation of the cell membrane and consequential decrease in the open probability of voltage-dependent Ca^{2+} channels (Quast et al., 1994). Thus, any change in the functional characteristics of K_{ATP} channels would alter the contractility of vascular smooth muscles.

In spontaneously contracting tissues like rat portal vein K_{ATP} channels have been reported to be the primary targets of K^+ channel openers. Activation of these channels by pinacidil and cromakalim is known to inhibit spontaneous rhythmic contractions. On the other hand, glibenclamide, a K_{ATP} channel blocker was shown to augment the rhythmic contractions and inhibit the relaxations caused by K^+ channel openers in portal vein preparations (Longmore et al., 1990). Using these K^+ channel ligands, the present study was undertaken to examine the modulation of vascular K_{ATP} channels and their involvement in causing reduced myogenic reactivity of the portal vein under hypothyroid state.

2. Materials and methods

2.1. Induction of hypothyroidism

Adult male albino rats (150–200 g) obtained from the Laboratory Animal Resource Section of this Institute, were

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used in the present study. Hypothyroidism was induced by 15 day treatment of rats with methimazole (10 mg/kg, i.p.; Swann, 1989). Control rats received comparable volume of normal saline, i.p. The thyroid states were determined by measuring plasma thyroxine (T_4) and triiodothyronine (T_3) levels in control and methimazole-treated rats by radioimmunoassay.

2.2. Tension experiments

Rats were stunned by a blow to the head and quickly exsanguinated. The hepatic portal vein was freed of the surrounding connective tissue and isolated. The portal vein was immediately transferred to oxygenated Tyrode's solution of the following composition (mM); NaCl, 120; KCl, 5.9; $NaHCO_3$, 11.9; $MgCl_2.6H_2O$, 1.2; $NaH_2PO_4.2H_2O$, 1.2; $CaCl_2.2H_2O$, 2.5 and glucose, 11.5.

The whole portal vein, approximately of 1.5 cm, was suspended in an organ bath containing 20 ml of Tyrode's solution, bubbled with O_2 (pH 7.4) at 37 ± 0.5°C under a resting force of 0.5 g and equilibrated for a period of 1 h. Isometric contractions were recorded by a force transducer connected to an ink-writing oscillograph (Recorders and Medicare, India).

2.3. Experimental protocol

2.3.1. Recording of spontaneous rhythmic contractions

After the equilibration period of 60 min, the spontaneous rhythmic contractions of the rat portal vein were recorded for a period of 5 min and the rate (contractions/min) and amplitude (g force) of myogenic activity were averaged and expressed as controls for each thyroid state.

2.3.2. Concentration-response curves of noradrenaline

Concentration-response curves were obtained for noradrenaline in portal veins taken from both euthyroid and hypothyroid rats. Log incremental concentrations of noradrenaline (10^{-9}–10^{-5} M) were added to the bathing solution, each one being 0.5 log unit higher than the preceding one. The absolute force development by the agonist was plotted against its log concentration to compare between euthyroid and hypothyroid states. Percentage conversion of the absolute force was done to determine the EC_{50} value of the agonist.

2.3.3. Concentration-response curves of K^+ channel openers

In order to examine the influence of the thyroid state on the sensitivity of the portal vein to K_{ATP} channel activators, pinacidil and cromakalim (Hamilton and Weston, 1989) were used to produce inhibition of rhythmic contractions in tissues obtained from both euthyroid and hypothyroid rats. A cumulative concentration-response curve for each K^+ channel opener was generated by adding the drug to the bath at an increment of one log unit in the absence or presence of glibenclamide (10^{-7} M). At each concentration of the vasodilator, a 5 min average of amplitude/frequency of myogenic contraction was taken following 5 min contact period of the drug. The antagonism by glibenclamide of the responses to pinacidil and cromakalim was studied using the following protocol. After the equilibration period of 60 min, the tissues were exposed to glibenclamide (10^{-7} M) for 15 min before the cumulative concentration-response curves to the vasodilators were obtained. The inhibitory responses of the tissue were expressed as a percentage of control in the absence of the drug. In some experiments, time-matched control responses to appropriate vehicles were obtained in tissues from both the groups.

2.3.4. Concentration-response curves for K_{ATP} channel blocker, glibenclamide

Concentration-response curves to K_{ATP} channel blocker, glibenclamide (Quast and Cook, 1989) were elicited in tissues obtained from both euthyroid and hypothyroid rats. The drug was added cumulatively at an increment of 0.5 log unit at an interval of 10 min. The effect of glibenclamide on the myogenic responses was expressed as a percentage increase in the rate and amplitude of contraction as compared to control responses (before exposure to the drug).

2.4. Drugs

The drugs used were: cromakalim (BRL 34915, Smith Kline and Beecham, UK) stock solution (10^{-3} M) in 70% ethanol, glibenclamide (Hoechst, UK) stock solution (10^{-2} M) in dimethyl sulfoxide, pinacidil (Leo, Denmark) stock solution (10^{-3} M) prepared in 0.1 N HCl and methimazole (Sigma, USA) 10 mg/ml dissolved in distilled water. The final concentration of the vehicles in the bathing solution did not exceed 1% (dimethyl sulfoxide 0.1% and ethanol 0.7%). A stock solution (10^{-3} M) of noradrenaline (Sigma) was prepared freshly in 0.1 N HCl.

2.5. Statistics

The results (absolute myogenic tone/percentage response) are presented as means ± S.E. Student's *t*-test was employed for the test of significance. The apparent potency of the drug (log EC_{50}/IC_{50}) was calculated by regression analysis and expressed as geometric mean with 95% confidence limits (Snedecor and Cochran, 1967).

3. Results

3.1. Assessment of thyroid status

In the hypothyroid animals, a significant ($P < 0.01$) decrease in plasma T_3 level (0.54 ± 0.04 ng/ml; $n = 6$)

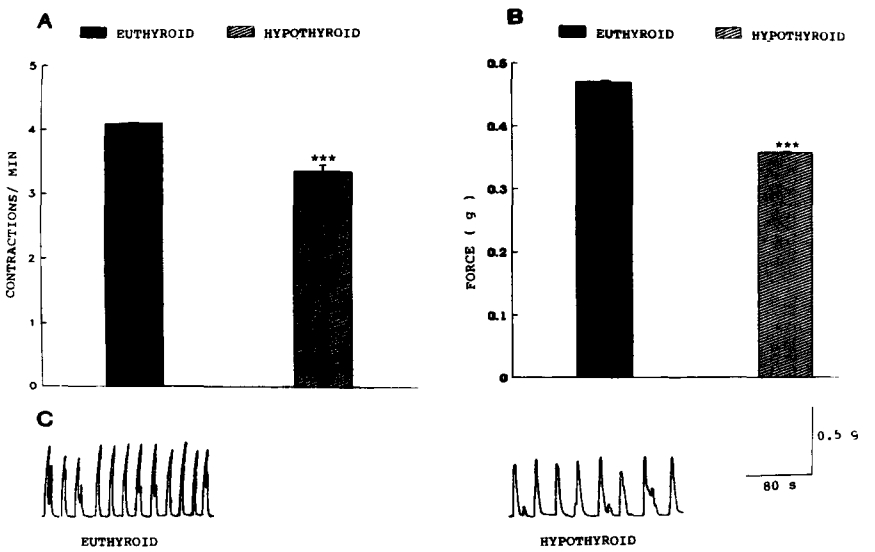

Fig. 1. The influence of hypothyroidism on spontaneous, rhythmic contractions of rat portal vein. The figure depicts the rate (A) and the amplitude (B) of rhythmic contractions in both euthyroid and hypothyroid states. The vertical bars represent the standard error of the mean (*** $P < 0.001$). The representative tracings show the spontaneous rhythmic contractions of portal vein taken from both euthyroid (left panel) and hypothyroid (right panel) rats.

was observed as compared to the saline-treated euthyroid controls (0.94 ± 0.04 ng/ml; $n = 6$). Similarly, a significant ($P < 0.01$) reduction in T_4 level occurred in hypothyroid rats (20.5 ± 1.52 ng/ml; $n = 6$) in comparison to controls (46.27 ± 1.52 ng/ml; $n = 6$).

3.2. Effect of hypothyroidism on spontaneous rhythmic contractions

Fig. 1 shows the influence of the hypothyroid state on the rate and amplitude of rhythmic contractions in the rat portal vein. The mean normal rate measured as contractions/min was 4.07 ± 0.01 ($n = 42$) in tissues from euthyroid rats, while it significantly ($P < 0.001$) decreased to 3.32 ± 0.08/min ($n = 42$) in hypothyroid state. Similarly, a significant ($P < 0.01$) reduction in the mean amplitude of spontaneous rhythmic contractions was observed in tissues taken from hypothyroid rats (0.36 ± 0.01 g; $n = 42$) as compared to that of the euthyroid controls (0.47 ± 0.02 g; $n = 42$). However, there was no significant difference in the mean contractile time (measured in respect of monophasic contractions) between euthyroid (5.82 ± 0.71 s; $n = 5$) and hypothyroid (5.31 ± 0.26 s; $n = 5$) states. With respect to time-matched controls, the amplitude and the frequency of the myogenic responses were 0.66 ± 0.01 g and 4.0 ± 0.01/min, respectively, at 0 min as compared to an amplitude of 0.63 ± 0.02 g and a frequency of 4.0 ± 0.01/min at the end of 90 min (the normal duration of the experiment) in tissues taken from euthyroid controls ($n = 3$). Similar observations were made in tissues obtained from hypothyroid rats.

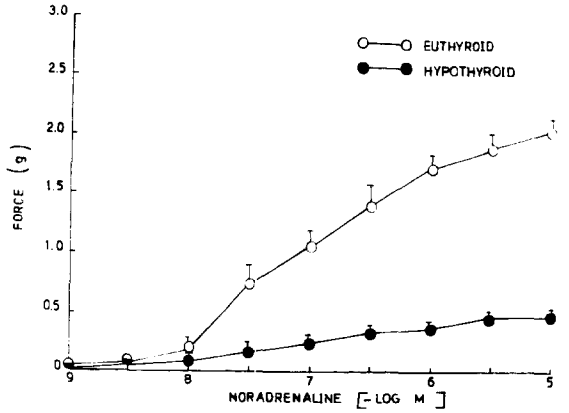

Fig. 2. Concentration-response curves of noradrenaline in rat portal veins in euthyroid ($n = 8$) and hypothyroid ($n = 6$) states. The vertical bars represent standard error of the mean.

3.3. Effect of hypothyroidism on the responses to noradrenaline

Fig. 2 shows the influence of the hypothyroid state on the contractile responses of the portal veins to noradrenaline. Noradrenaline (10^{-9}–10^{-5} M) elicited concentration-related contractions in tissues obtained from both the groups. However, as can be seen from the concentration-response curves, the contractile responses to noradrenaline at any given concentration ($> 10^{-8}$ M) were markedly blunted in the preparations from the hypothyroid rats as compared to euthyroid controls. Thus, in the euthyroid state the maximum absolute force generated by noradrenaline (10^{-5} M) was 2.05 ± 0.1 g ($n = 8$) which was significantly ($P < 0.01$) greater than the maximum abso-

lute force (0.45 ± 0.01 g; $n = 6$) obtained at similar concentration of the agonist in hypothyroid condition. The EC_{50} values of noradrenaline in the controls (2.04×10^{-7} M; 95% CL, $0.83–2.75 \times 10^{-7}$ M; $n = 8$) were, however, not significantly different from the hypothyroid state (0.69×10^{-7} M; 95% CL, $0.35–1.09 \times 10^{-7}$ M; $n = 6$).

3.4. Sensitivity of rhythmic contractions to pinacidil and cromakalim

The inhibitory concentration-response curves for pinacidil and cromakalim in the presence and absence of K^{ATP} channel blocker glibenclamide (10^{-7} M) are shown in Fig. 3. Pinacidil ($10^{-9}–10^{-5}$ M) added cumulatively produced concentration-related inhibition in the amplitude of spontaneous rhythmic contractions of portal veins from both euthyroid and hypothyroid rats with complete inhibition occurring at 10^{-5} M and 10^{-6} M, respectively. However, the tissues obtained from the hypothyroid rats exhibited greater sensitivity to pinacidil as compared to the controls (Fig. 3A). Thus, a significant decrease in the IC_{50} value of pinacidil was noted in the hypothyroid state (0.92×10^{-8} M; 95% CL, $0.79–1.0 \times 10^{-8}$ M; $n = 6$) as compared to the euthyroid controls (5.1×10^{-8} M; 95% CL, $4.17–6.03 \times 10^{-8}$ M; $n = 6$). When the tissues were exposed to glibenclamide (10^{-7} M) for 15 min before the addition of the vasodilator, the frequency of myogenic tone was increased by approximately 25% and 40% in euthyroid and hypothyroid states, respectively. Glibenclamide caused a rightward parallel shift in the concentration-response curves of pinacidil with corresponding increase in the IC^{50} values of the vasodilator both in euthyroid (2.04×10^{-7} M; 95% CL, $0.55–4.4 \times 10^{-7}$ M; $n = 6$) and hypothyroid (0.49×10^{-7} M; 95% CL, $0.31–0.79 \times 10^{-7}$ M; $n = 6$) states, respectively.

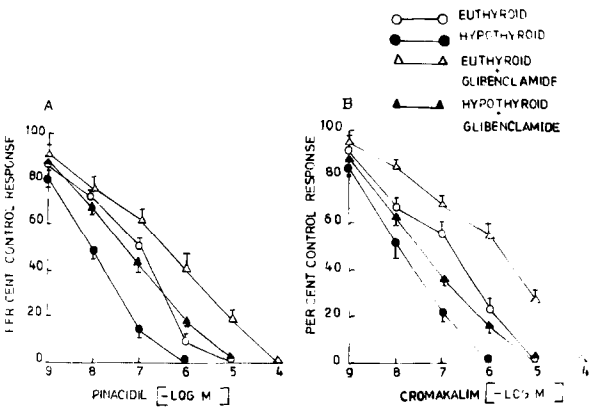

Fig. 3. The influence of hypothyroidism on the sensitivity of the portal vein to potassium channel openers. The figure depicts concentration-dependent inhibition in the amplitude of myogenic tone by pinacidil (A) and cromakalim (B), in the absence and presence of glibenclamide (10^{-7} M). The vertical bars represent standard error of the mean ($n = 6$ for each group).

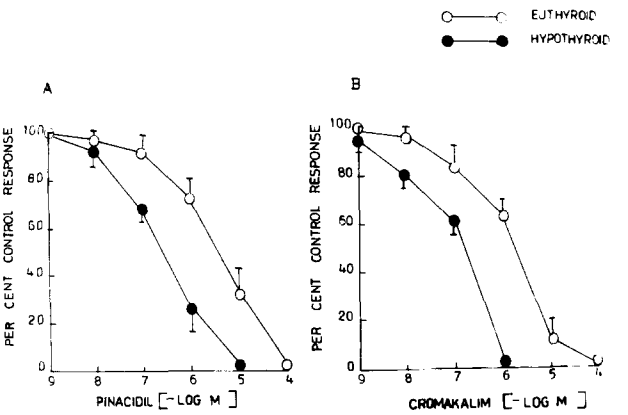

Fig. 4. Effect of hypothyroidism on concentration-dependent inhibition in the frequency of spontaneous rhythmic contractions by pinacidil (A) and cromakalim (B) in the presence of glibenclamide (10^{-7} M). The vertical bars represent standard error of the mean ($n = 6$ for each group).

Similar to the amplitude, the frequency of myogenic tone of the tissues obtained from hypothyroid rats showed greater sensitivity to pinacidil. Thus, a significant decrease in the IC_{50} value of pinacidil was observed in the hypothyroid condition (1.08×10^{-7} M; 95% CL, $0.46–2.49 \times 10^{-7}$ M; $n = 5$) in comparison to controls (5.56×10^{-7} M; 95% CL, $3.66–8.43 \times 10^{-7}$ M; $n = 6$). Glibenclamide (10^{-7} M) caused a rightward shift in the concentration-response curves of pinacidil in both the groups (not shown). The results of pinacidil in the presence of glibenclamide are depicted in Fig. 4A. As can be seen here, the inhibitory concentration-response curve to pinacidil was shifted to the left in the hypothyroid state with a corresponding decrease in its IC_{50} (2.08×10^{-7} M; 95% CL, $1.07–4.06 \times 10^{-7}$ M; $n = 6$) when compared with the controls (2.05×10^{-6} M; 95% CL, $0.98–4.29 \times 10^{-6}$ M; $n = 6$).

As observed with pinacidil, a marked increase in the sensitivity of the myogenic tone (both frequency and amplitude) to cromakalim was evident in hypothyroid condition (Fig. 3B and Fig. 4B). The results in respect of concentration-dependent inhibition in the amplitude of myogenic contractions by cromakalim ($10^{-9}–10^{-5}$ M) are presented in Fig. 3B. A significant ($P < 0.05$) decrease in the IC_{50} value of cromakalim was noted in the hypothyroid state (0.12×10^{-7} M; 95% CL, $0.07–0.19 \times 10^{-7}$; $n = 6$) as compared to the euthyroid state (0.78×10^{-7} M; 95% CL, $0.42–1.12 \times 10^{-7}$ M; $n = 6$). Pre-treatment of the tissues for 15 min with glibenclamide (10^{-7} M) produced a parallel shift to the right of the inhibitory concentration-response curves of cromakalim in both the thyroid states. Thus, the IC_{50} values of the K^+ channel opener in the presence of glibenclamide were 6.31×10^{-7} M (95% CL, $4.07–7.94 \times 10^{-7}$ M; $n = 6$) and 0.39×10^{-7} M (95% CL, $0.34–0.47 \times 10^{-7}$ M; $n = 6$), in euthyroid and hypothyroid states, respectively.

As regards the rate of rhythmic contractions of the portal vein hypothyroidism caused a leftward shift in the

inhibitory concentration-response curve to cromakalim with the corresponding decrease in the IC_{50} value (4.3×10^{-8} M; 95% CL, $0.17–1.05 \times 10^{-7}$ M; $n = 5$) of the vasodilator as compared to control (3.02×10^{-7} M; 95% CL, $1.06–4.61 \times 10^{-7}$ M; $n = 6$). Glibenclamide (10^{-7} M) caused parallel rightward shift of the concentration-response curves of cromakalim in both the groups (not shown). Fig. 4B presents the results of cromakalim in the presence of glibenclamide. As can be noted here, there was a leftward shift in the concentration-response curve to the vasodilator associated with a significant ($P < 0.05$) decrease in the IC_{50} value in hypothyroid state (7.4×10^{-8} M; 95% CL, $0.47–1.16 \times 10^{-7}$ M; $n = 6$) when compared with euthyroid controls (2.0×10^{-6} M; 95% CL, $1.29–3.11 \times 10^{-6}$ M; $n = 6$). In an experiment on the portal vein taken from the euthyroid control, the amplitude and the frequency of the myogenic responses were 0.5 g and 4.0/min, respectively before and 0.47 g and 4.0/min after exposure to the vehicle, ethanol (0.7%, the highest concentration in the bath) used to dissolve cromakalim.

3.5. Effect of glibenclamide on the myogenic responses of the portal vein

Fig. 5 shows the concentration-response (percentage increase in rate of myogenic contractions) relationships observed on cumulative application of various concentrations of glibenclamide ($10^{-8}–3 \times 10^{-6}$ M) in portal veins

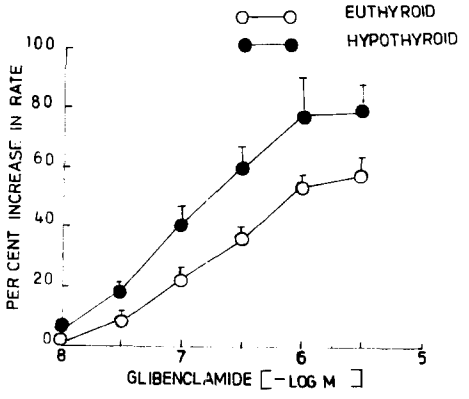

Fig. 5. Concentration-dependent increase in the frequency of myogenic tone by glibenclamide in portal veins taken from both euthyroid and hypothyroid rats. The vertical bars represent standard error of the mean ($n = 4$ for each group).

obtained from both euthyroid and hypothyroid rats. The EC_{50} value of the K_{ATP} channel blocker was significantly ($P < 0.05$) less in portal veins from hypothyroid rats (2.07×10^{-7} M; 95% CL, $1.06–4.05 \times 10^{-7}$ M; $n = 4$) as compared to euthyroid controls (8.07×10^{-7} M; 95% CL, $0.53–1.22 \times 10^{-6}$ M; $n = 4$). As regards the amplitude of the myogenic responses, glibenclamide (10^{-8}, 3×10^{-8} and 10^{-7} M) caused a concentration-dependent increase in the force of contraction in tissues taken from both euthyroid and hypothyroid rats (Table 1). The maximum percentage increase in the amplitude of myogenic contractions by glibenclamide at 10^{-7} M was 51.0 ± 3.0 ($n = 4$) and 45.0 ± 7.0 ($n = 4$) in euthyroid and hypothyroid states, respectively. Further increase in the concentration of glibenclamide between 3×10^{-7} M and 3×10^{-6} M did not cause an increase in the amplitude of contractions. On the contrary, there was a decline in the maxima. The vehicle, dimethyl sulfoxide, at the highest concentration (0.1%) used in the bath caused 5% reduction in the amplitude while it had no effect on the frequency of the spontaneous contractions in both the states.

4. Discussion

A decrease in the rate and amplitude of spontaneous rhythmic contractions in portal veins from hypothyroid rats, as observed in the present study, is consistent with an earlier observation wherein methimazole-induced hypothyroidism was shown to reduce the spontaneous myogenic tone of this preparation (Chin and Pennefather, 1992). In spontaneously active vascular smooth muscles, K^+ channels are thought to determine the resting membrane potential and firing frequencies of the pace maker cells which, in turn, influence the myogenic activity (Longmore and Weston, 1990; Cavero and Guillon, 1993). Therefore, any intervention that either augments or inhibits the opening of K^+ channels should also alter the myogenic activity of the tissue. Keeping this in view, the modulation of K_{ATP} channels in hypothyroidism was studied using the rat portal vein as a model of vascular smooth muscle.

Evidence for the presence of K_{ATP} channels in portal vein is obtained from tension experiments on whole tissue (Winquist et al., 1989; Wickenden et al., 1991) and patch clamp studies on single vascular myocytes (Kajioka et al.,

Table 1
Effect of glibenclamide ($10^{-8}–3 \times 10^{-6}$ M) on the amplitude of the myogenic activity of rat portal vein (expressed as percentage increase)

Treatment	Concentration (M)					
	10^{-8}	3×10^{-8}	10^{-7}	3×10^{-7}	10^{-6}	3×10^{-6}
Euthyroid	22.0 ± 1.5	43.3 ± 4.8	51.0 ± 3.0	24.0 ± 10.0	15.5 ± 10.0	0.0
Hypothyroid	19.0 ± 2.08	33.0 ± 7.0	45.0 ± 7.0	12.7 ± 8.2	3.7 ± 3.7	0.0

$n = 4$ in each group.

1990; Noack et al., 1992). The inhibition of rhythmic contractions of portal veins, taken from both euthyroid and hypothyroid rats by K^+ channel openers at pharmacologically relevant concentrations appears to be mediated by the activation of vascular K_{ATP} channels. The involvement of these channels is further substantiated by the observation that glibenclamide antagonised the mechano-inhibitory responses of pinacidil and cromakalim in this tissue. Previous studies demonstrated that like many other vascular smooth muscles (Standen et al., 1989; Quast et al., 1994), relaxation caused by K^+ channel openers and their antagonism by glibenclamide in portal vein involves K_{ATP} channels (Winquist et al., 1989; Longmore et al., 1990).

One of the most important observations of the present study is that there was a significant increase in the reactivity of the portal vein to K^+ channel openers in tissues from hypothyroid rats. This was evident from a leftward shift in the concentration-response curves of pinacidil and cromakalim with a corresponding decrease in their IC_{50} values. A similar phenomenon was also observed with glibenclamide which produced a greater increase in the frequency of the myogenic tone in the hypothyroid state. These observations suggest that the enhanced opening of K_{ATP} channel is possibly related to an alteration in the metabolic state of the vascular smooth muscle in hypothyroidism. It is well established that while thyroid hormones enhance rate of oxidative phosphorylation, thereby causing increased synthesis of ATP, an opposing effect is observed in hypothyroidism as indicated by a lower energy production (Guyton, 1990). Metabolic depletion of ATP in rat portal vein has been reported to inhibit the spontaneous myogenic tone which could be restored by glibenclamide (Longmore et al., 1990). It is, therefore, presumed that the changes in ATP-ADP ratio in hypothyroidism would influence the responses of the tissue to the K^+ channel ligands acting on K_{ATP} channels.

Besides the myogenic tone, the influence of hypothyroidism on agonist-induced contractions in rat portal vein was also assessed. There was not only reduction in myogenic tone but contractions evoked by noradrenaline were also markedly depressed in portal veins obtained from hypothyroid rats. This observation is consistent with an earlier observation wherein blunting of contractile responses to noradrenaline in hypothyroidism was shown in rat aorta (Gunasekera and Kuriyama, 1990). Interestingly, in their studies, the inhibition of mechanical responses to noradrenaline could not be correlated to an increased number of α_1-adrenoceptors in aortae from hypothyroid rats. Hence, the inhibitory effect of membrane hyperpolarisation, as a result of increased K_{ATP} channel opening, on the contractions elicited by noradrenaline cannot be ruled out. Membrane hyperpolarisation has been shown to inhibit agonist-induced synthesis of inositol 1,4,5-triphosphate with a consequential inhibition in the release of intracellular Ca^{2+}, thus restraining the contractile responses to agonist in vascular smooth muscles (Itoh et al., 1992; Quast et al., 1994).

In conclusion, the observations made in the present study suggest that hypothyroidism-induced decrease in portal venous tone is associated with an enhanced opening of the K_{ATP} channels.

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