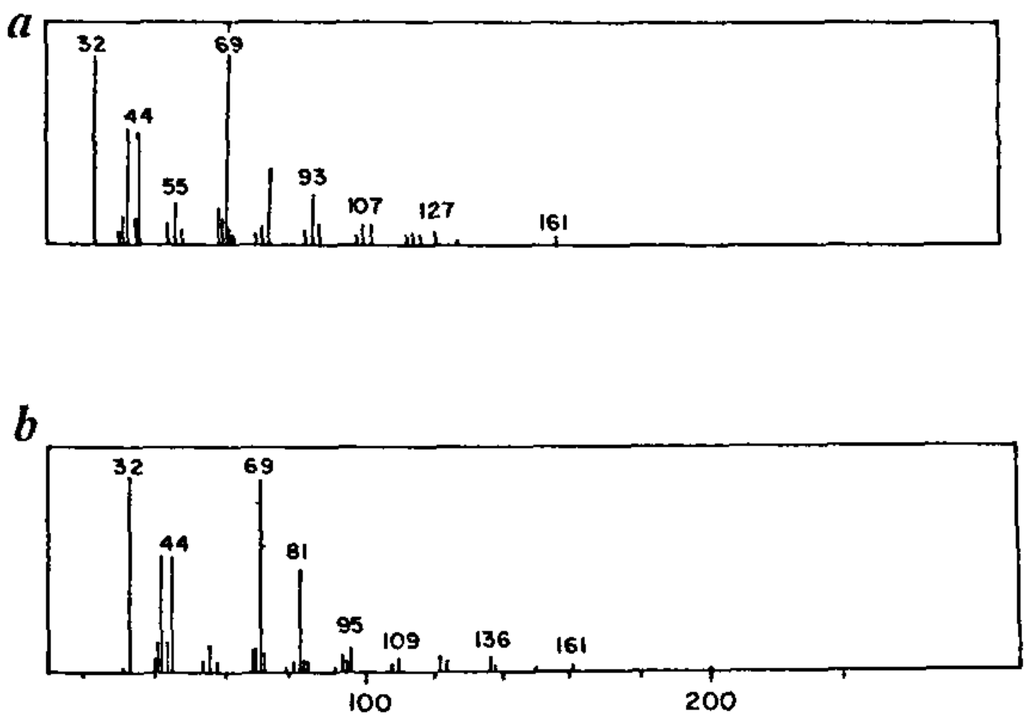

Figure 4. Electron impact-mass spectra of (*a*) Compound I (2,6,10-dodecatrien-1-ol,3,7,11-trimethyl (Z, E) and (*b*) standard for compound I isolated from male preputial gland.

opposite sexes, 3 animals were used in each set for behaviour analysis. Fresh samples were used in each trial. The behaviour was assessed for 15 min with these compounds and the solvents alone as control. The responders were of the same and the opposite sex. The time taken for visiting each fraction was recorded and subjected to statistical analyses.

The extracted male preputial gland contains 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl (Z, E), di-*n*-octyl phthalate and 1,2,benzenedicarboxylic acid, diisooctyl ester (Figures 1–4). These chemical substances have their unique functions. The first two compounds are involved in the attraction of the opposite sex and the third compound is involved in the attraction of the same sex (Table 1). Thus, the preputial gland of male rat contains three different compounds with distinct social functions.

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Effect of chronic atenolol treatment on responses to noradrenaline and terbutaline in isolated ventricle from hypertensive and hyperthyroid rats

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The present investigation was undertaken to study the effect of chronic treatment with atenolol on noradrenaline and terbutaline in hypertensive and hyperthyroid rats. The maximum increase in force of contraction of ventricle by noradrenaline in control preparations chronically treated with atenolol was reduced, while that produced by terbutaline was not affected. The pD_2 values of both the agonists were increased. In DOCA-saline hypertensive preparations, chronic treatment with atenelol did not produce any change in noradrenaline-induced positive inotropic responses; however, the maximal response to terbutaline was reduced. While the pD_2 value of noradrenaline was decreased, that of terbutaline was increased. Chronic treatment with atenolol in hyperthyroid rats did not produce any significant effect on the maximal contractions of ventricle with either noradrenaline or terbutaline. However, the pD_2 value of noradrenaline was reduced while that of terbutaline was unaffected. It is concluded that the beneficial effects of atenolol in hypertension and hyperthyroidism may be related to reduction in the number of beta-receptors in the heart.

BETA$_1$- and beta$_2$-adrenoceptors co-exist in the hearts of most species, with the beta$_1$-adrenoceptor predominating functionally under normal physiological conditions[1–3]. Chronic administration of agonist or antagonist receptor ligands results in decrease (down-regulation) and increase (up-regulation) respectively in ligand-binding

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densities[4]. Prolonged treatment with beta-receptor blocking agent, propranolol, can lead to an increase in the beta-receptor number in various tissues, which may contribute to the development of withdrawal symptoms if discontinuation of drug therapy is abrupt[5]. However beta-adrenoceptor up-regulation is not an automatic consequence of antagonist exposure and in some systems beta-adrenoceptor decreases[6]. This down-regulation may contribute to the pharmacological activity of these agents, but the mechanism of action remains to be defined[7]. Cardiac beta-adrenoceptor function may be altered in hypertension[8]. The decrease in beta-functions response may be attributed partly to the down-regulation of $beta_1$-adrenoceptors in hypertension. Little is known about functional $beta_2$-adrenoceptors in hypertension[9]. Clinically hyperthyroidism may be associated with systolic hypertension. The intention of the present study was to see if the hypertension or hyperthyroid-state-induced changes in responses to agonists could be reversed by chronic treatment with atenolol.

Male Wistar rats (250–350 g) were sacrificed by a sharp blow on the head and cutting of the neck blood vessels. The heart was rapidly removed and placed in a petri dish containing oxygenated, prewarmed (35°C) Ringer Locke physiological salt solution of the composition (mM): NaCl – 15.4; KCl – 5.6; $CaCl_2$ – 2.2; $NaHCO_3$ – 6.0; glucose – 11.1. The solution always contained guanethidine (1×10^{-5} M) and atropine (1×10^{-6} M). The free wall of the right ventricle was excised and the intact right ventricle was suspended[10] 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, during which the solution was changed every 15 min, the ventricle was driven by square wave pulses (5 PPS, 5 ms 1 ms delay, supramaximal voltage 50 V). The electrodes were attached to stimulator research model SS-48 (Recorders and Medicare System, Chandigarh). Isometric tension was recorded using the force displacement transducer (Ft 1312).

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

Groups of 5–10 normal or 3–4 hypertensive rats received similar chronic treatments with atenolol (3 mg/kg) dissolved in triple-glass-distilled-water and administered orally via a Ryles tube once daily for 28 days[11]. Another group of rats received simultaneously the same dose of L-thyroxine (0.75 mg/kg in 0.001 N NaOH in 0.9% NaCl; subcutaneously) and atenolol (3 mg/kg dissolved in triple-glass-distilled-water orally via a Ryles tube) once daily for 7 days.

Male albino rats weighing about 100 g were kept on a diet high in sodium chloride and drinking water was replaced by 2% sodium chloride solution *ad lib*. When the rats attained a weight of about 250 g, they were given deoxycorticosterone acetate (DOCA) dissolved in sesame seed oil in a dose of 10 mg/kg, subcutaneously twice weekly for 42 days[12].

To check whether hypertension had been produced by the DOCA-saline treatment schedule, blood pressure of rats was recorded[13]. Following confirmation of induction of hypertension, groups of 3–4 rats received chronic treatment with drugs as discussed above.

Hyperthyroidism was induced by subcutaneous injection of 0.75 mg/kg L-thyroxine sodium in alkaline saline solution (0.001 N NaOH in 0.9% NaCl) daily for 7 days[14].

Rectal temperature and heart weights of L-thyroxine-treated animals were recorded before the start of the treatment and before sacrifice. Serum was prepared from blood samples collected from the common carotid artery of rat exsanguinated on the day of the experiment and stored at –20°C. Total serum thyroxine (T_4), tri-iodothyronine (T_3) and thyroxine stimulating hormone (TSH) levels were determined by enzyme immunoassay (EIA) with a commercially available *in vitro* 'diagnostic kit' (Bio Me'rieux, France) on semi-autoanalyser (SEAC CH-100; Ames marketed by Miles India, Baroda).

Microscopic examination of the ventricular tissue was performed according to the method of Helper[15].

Noradrenaline (± arterenol; NA); atropine sulphate and guanethidine (1-[2-guanidinoethy1] octa hydroazocine) monosulphate were obtained from Sigma. Terbutaline was obtained form Astra–IDL. The following drugs were received as free gifts; atenolol (Cadila, Ahmedabad); deoxycorticosterone acetate (Infar, Bombay); L-thyroxine sodium (Glaxo, Calcutta).

Polyethylene glycol 400 (E. Merck, Bombay), glycerine I. P. (Metro, Wadhwan City), sesame seed oil (Ahmed Mills, Bombay) were obtained and used as solvents of the drugs.

Only one agonist was used for getting concentration-response curve in a given preparation. Contractile force was expressed as gram of tension developed. The pD_2 values were determined from the EC_{50} (ref. 16). The results are expressed as mean ± SEM and analysed by the Student's *t* test for obtaining the level of significance[17].

Noradrenaline (3.51×10^{-7} M to 1.4×10^{-6} M) and terbutaline (3.03×10^{-6} M to 9.11×10^{-6} M) produced concentration-dependent increase in the force of contraction in electrically driven isolated right ventricle. The maximum increase in force of contraction by noradrenaline in control preparations chronically treated with atenolol was found to be reduced significantly ($P < 0.01$). The response to terbutaline was, however, not altered significantly. The pD_2 values of both the agonists were increased ($P < 0.01$) (Table 1).

Table 1. Effect of atenolol on the maximal contractile force and pD_2 values obtained in rat isolated ventricle with noradrenaline and terbutaline in control, DOCA-saline hypertensive and hyperthyroid rats

	Noradrenaline			Terbutaline		
Treatment group	Maximum contractile force (g)	*n*	pD_2 value	Maximum contractile force (g)	*n*	pD_2 value
Control	4.07 ± 0.19	6	6.33 ± 0.04	0.81 ± 0.14	5	5.26 ± 0.01
Chronic atenolol	2.79 ± 0.11*	5	6.94 ± 0.01*	0.99 ± 0.09^{NS}	3	6.32 ± 0.02*
DOCA-saline	3.96 ± 0.19^{NS}	3	6.90 ± 0.02*	2.07 ± 0.01*	3	5.40 ± 0.06*
DOCA-saline + chronic atenolol	4.02 ± 0.08^{NS}	3	6.45 ± 0.04*	1.74 ± 0.10**	3	5.74 ± 0.03*
L-thyroxine	2.01 ± 0.16*	3	7.00 ± 0.00*	1.74 ± 0.11*	3	5.55 ± 0.07*
L-thyroxine + chronic atenolol	2.07 ± 0.00^{NS}	3	6.32 ± 0.00*	1.92 ± 0.03^{NS}	3	5.66 ± 0.06^{NS}

Values are listed as ± SEM

*$P < 0.01$ and **$P < 0.05$, significant difference; NS, no significant difference. Comparisons are made between (i) chronic atenolol/DOCA-saline/L-thyroxine and control (ii) chronic DOCA-saline and chronic DOCA-saline + atenolol (iii) L-thyroxine and chronic L-thyroxine + atenolol.

Table 2. Effect of chronic treatment with atenolol on various somatic parameters measured following altered thyroid state in rats

		Treatment group	
Variable	Control	L-thyroxine	L-thyroxine + atenolol
Body weight (g)	250.00 ± 0.00	216.66 ± 10.54*	250.00 ± 12.91**
Rectal temperature in °C	37.81 ± 0.03	39.56 ± 0.15*	38.34 ± 0.11*
Heart weight (mg 100 g^{-1} body wt.)	362.00 ± 7.00	594.00 ± 1.80*	518.00 ± 29.2*
Serum thyroxine (T_4) (mcg %)	11.3 ± 0.30	38.26 ± 0.35*	36.33 ± 1.43^{NS}
Serum tri-iodothyronine (T_3) (ng/ml)	1.23 ± 0.08	8.68 ± 0.50*	7.76 ± 0.10**
Serum TSH (μ/ml)	3.83 ± 0.03	0.24 ± 0.02*	0.10 ± 0.00^{NS}

Values are listed as ± SEM

*$P < 0.01$ and **$P < 0.05$, significant difference; NS, no significant difference. Comparisons are made between (i) chronic L-thyroxine and control (ii) chronic L-thyroxine treatment and chronic L-thyroxine + atenolol treatment.

In DOCA-saline hypertensive preparations, noradrenaline-induced increase in force of contraction was not altered significantly, while terbutaline showed a significant increase compared to control. The pD_2 values of both the agonists showed an increase compared to the control. Chronic treatment with atenolol did not produce any change in noradrenaline-induced positive inotropic responses, however, the maximum positive inotropic effect of terbutaline was reduced ($P < 0.05$) compared to DOCA-saline control. The pD_2 value of noradrenaline showed a decrease while pD_2 value of terbutaline showed an increase ($P < 0.01$) (Table 1).

Hyperthyroid state in rats produced significant increase in heart weight and hyperthermia (Table 2). Serum T_3 and T_4 levels were increased while TSH level was reduced significantly ($P < 0.01$). Chronic treatment with atenolol produced significant ($P < 0.01$) reduction in the heart weight and rectal temperature, without affecting T_4 and TSH levels. There was, however, slight but significant decrease in T_3 level after atenolol treatment (Table 2).

Chronic treatment with L-thyroxine reduced ($P < 0.01$) the maximal contraction with noradrenaline and increased that with terbutaline; the pD_2 values were increased ($P < 0.01$) with both the agonists. Chronic treatment with atenolol in hyperthyroid rats did not produce any significant effect on the maximal contractions of ventricle with noradrenaline and terbutaline. However the pD_2 value of noradrenaline was reduced ($P < 0.01$), while that of terbutaline was unaffected (Table 1).

Microscopic examination of stained ventricular tissues of DOCA-saline-treated rats showed focal aggregates of inflammatory cells (b). Atenolol treatment had no effect in some (cc_1) and restored the profile in others (cd_2) (Figure 1).

The results confirm the co-existence of $beta_1$- and $beta_2$-adrenoceptors in rat ventricle. This also strongly supports the view that in the rat right ventricle, as in human hearts, $beta_2$-adrenoceptors can mediate a positive inotropic effect, but these effects are smaller compared to $beta_1$-adrenoceptor-mediated effects[18].

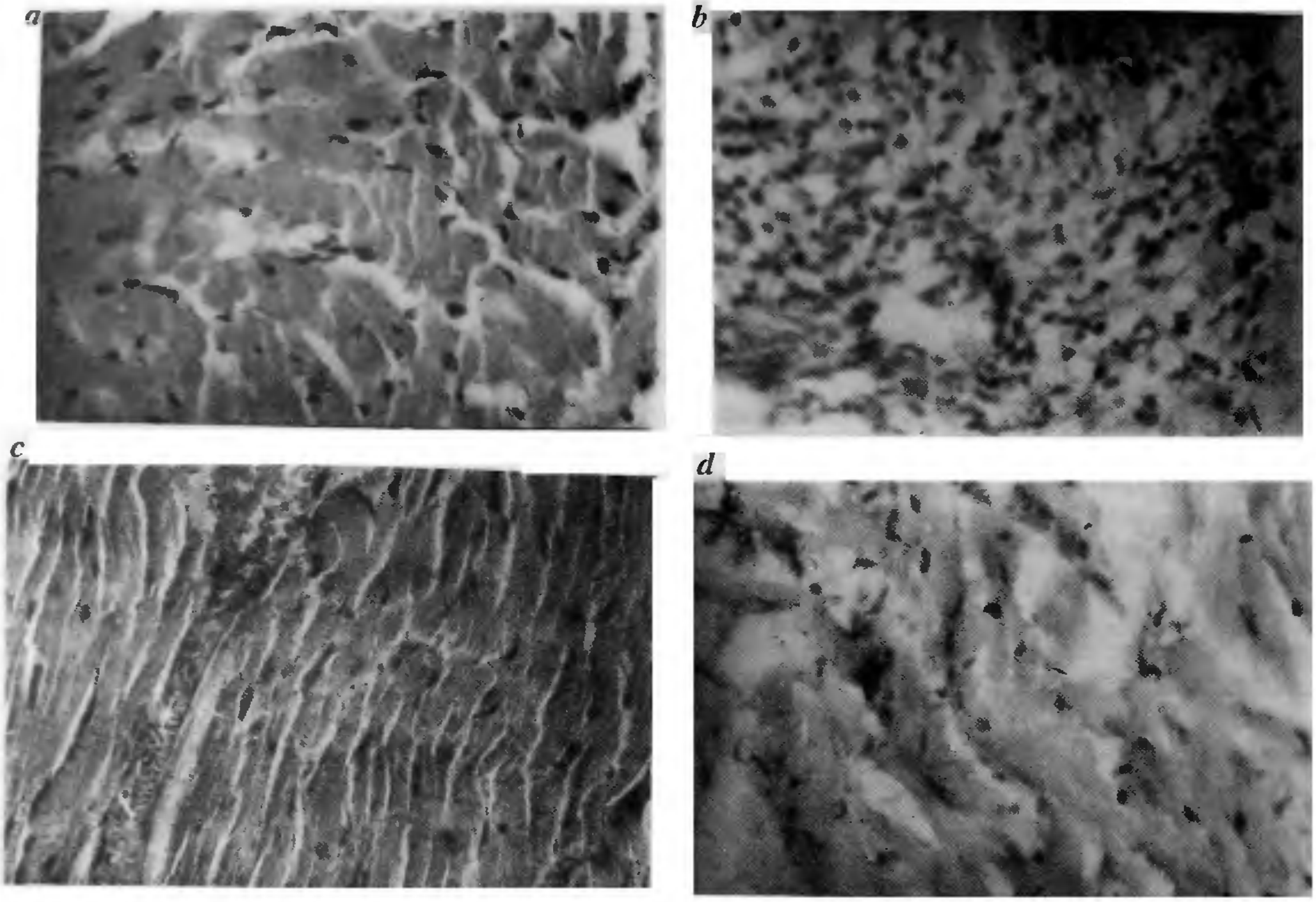

Figure 1. Photomicrograph (×47) of the cut section of the right ventricle from (*a*), normotensive rat depicting normal structure; (*b*), rat chronically treated for 42 days with DOCA-saline. The photomicrograph depicts focal aggregates of inflammatory cells; (*c*, *d*), in rats chronically treated for 42 days with DOCA-saline and then with atenolol for 28 days. The photomicrograph depicts (*c*) congestion of vessels (*d*) reversal of pathology to normal.

Atenolol, at a concentration which blocks $beta_1$-adrenergic activity, results in virtually no $beta_2$-antagonism[19,20]. In the present study chronic atenolol treatment increased the pD_2 values of both noradrenaline and terbutaline, suggesting an increase in the beta-adrenoceptor density. The decrease in the maximal response with noradrenaline and no effect on the response to terbutaline may be due to down-regulation of the $beta_1$-receptors with compensatory up-regulation of the $beta_2$-receptors.

In hypertensive preparations there was an increase in the pD_2 value of noradrenaline, implying increased beta-adrenoceptor density; the maximal response was not changed. However with terbutaline, there was an increase in both the pD_2 value and the maximal response, suggesting an increase in the $beta_2$ receptors and increased calcium influx[21]. Chronic treatment with atenolol reduced the pD_2 value of noradrenaline with no change in the maximal contractile force of ventricle, however, the pD_2 value of terbutaline was increased with a decrease in the maximal contraction, possibly due to depletion of noradrenaline at the sympathetic terminal by interfering with transmitter turnover[22] or uptake[23] or both. Reduction or reversal by atenolol of the process of arterial hypertrophy or hyperplasia associated with hypertension may be an alternate possibility[24]. The restoration of tissue damage detected microscopically in some atenolol-treated preparations from hypertensive rats supports the above suggestion. Further work with specific radioligands would be necessary to confirm this suggestion.

Chronic L-thyroxine treatment increased the pD_2 value of noradrenaline and reduced the maximal response, implying increased beta-adrenoceptor density and decreased calcium influx. However with terbutaline, there was significant increase in the pD_2 value together with increased calcium influx. Simultaneous chronic treatment with L-thyroxine and atenolol decreased the pD_2 value of noradrenaline with no change in the pD_2 value of terbutaline and in the maximal contractile force of ventricle, suggesting that this may be due to a slight reduction in the $beta_1$-adrenoceptor with compensatory

increase in the $beta_2$-receptors. Apart from this, the significant somatic changes seen in rats treated simultaneously with L-thyroxine and atenolol, specially the heart weight and T_3 levels, may have played some vital role in the decrease of the affinity (pD_2 value) of adrenoceptors to noradrenaline.

It is concluded that the beneficial effects of atenolol in hypertension and hyperthyroidism may be related to the reduction in the number of $beta_1$-adrenoceptors in heart.

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Octopamine titer in the circulating fluid of tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) and its response to injected estrogen during critical phase of diapause termination

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The presence and role of the biogenic amine, octopamine has been demonstrated in *Antheraea mylitta* during pupal diapause. For elucidation of estrogen-induced responsiveness of insects, three consecutive injections of estradiol-17-β (E_2) at doses of 1, 5, 10 and 50 μg/pupa on days 130, 135, and 140 of pupal age were injected to both male and female pupae of *A. mylitta* during diapause. E_2 treatment caused a significant enhancement in plasma octopamine concentration of haemolymph on day 150 (except in males with 50 μg dose) and reduction on day 165 in both the male and female *A. mylitta*. On the contrary, plasma protein concentration was found to be higher only on day 150 when treated with E_2 between 1 and 50 μg doses. Octopamine titer in haemolymph plasma always remained higher in male than its female counterpart while in case of plasma protein titer it was found to be just reverse in control animals. E_2, @1–50 μg/pupa caused a significant reduction in pupal duration inducing early moth eclosion. Egg production increased at lower doses and decreased at higher doses of this hormone. E_2 at the dose by 0.5 μg/pupa remained ineffective in all the cases except in elevating the female plasma protein titer and egg production. Hence, diapausing pupae of tropical tasar silkworm, *A. mylitta* is physiologically responsive to vertebrate estrogen, E_2.

PUPAL diapause is a common phenomenon in wild tropical tasar silkworm *Antheraea mylitta* Drury, which continues up to 200–210 days depending on the ambient environmental conditions[1]. In Lepidoptera, pupal diapause occurs because the pupal brain stops secreting the peptidic prothoracicotropic hormone (PTTH) in response to diapause programming signals (mainly short day photoperiod) received in the larval stage. According to Denlinger[2], since PTTH is necessary for maintaining

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