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A study on α-adrenoceptor mediated contractile responses of high fat diet fed rat thoracic aorta

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Feeding rats with high fat diet (HFD) leads to the various conditions of syndrome-X. These are associated with hypertension through a variety of mechanisms. Vascular abnormalities probably contribute to the etiology of many diabetic complications. There is an increase in maximal responses to various agonists with blood vessels of streptozotocin induced diabetic animals. The purpose of this study was to evaluate the development in HFD fed rats for altered biochemical parameters, to assess the vascular responses to phenylephrine (PE), to estimate the K_A values and to observe the receptor occupancy. Body weights, plasma triglycerides, cholesterol, and glucose levels were measured every week in male Sprague-Dawley rats. Glucose tolerance test was performed after 4 weeks of feeding. At the end of the fourth week of feeding, concentration-response curves of PE were recorded. Altered K_A values of PE (NPD fed rats 2.0 ± 0.4 μM and HFD fed rats 0.3 ± 0.1 μM) and receptor occupancy response (NPD fed rats 92.1 ± 1.7% and HFD fed rats 77.5 ± 5.6%) strongly suggest that hypertension in HFD fed rats is associated with altered α-adrenoceptor function.

1. Introduction

Diabetes mellitus, hypertension, dyslipidemia and obesity are interrelated and characterize Syndrome-X or insulin resistance syndrome (Amos et al. 1997). Insulin resistance is one of the main factors in Non Insulin Dependent Diabetes Mellitus (NIDDM), which is associated with dyslipidemia (Arner 2002). There may be different vascular complications arising while the disease progresses, such as large (macrovascular), small vessel (microvascular) or microangiopathic and neuropathic diseases (which have a micro vascular component) (Bate and Jerums 2003).

Patients with diabetes mellitus are more prone to hypertension (Greenfield and Chisholm 2000). The mortality rate due to cardiovascular complications is two times higher in diabetic patients (Fisher 2003). Altered vascular reactivity is observed in various chemically induced diabetic animal models (MacLeod 1985). The mechanisms involved are not completely understood and remain unexplored. These enhanced vascular responses may be due to enhanced α-adrenoceptor sensitivity and number (Hodgkin et al. 1991), endothelial dysfunction (Reil et al. 1999; Steinberg et al. 1996) or alteration in signaling (Abebe et al. 1994; Abebe and MacLeod 1990; Sterin-Borda et al. 1984; White and Carrrier 1988).

The streptozotocin (STZ) induced diabetes model shows similar conditions of Insulin Dependent Diabetes Mellitus (IDDM). High fat diet (HFD) fed animals show conditions similar to those existing in NIDDM patients such as hyperglycemia, hypercholesterolemia, and hypertriglyceridemia (Han et al. 1997; Verwaerde et al. 1996). The HFD fed rat model is selected for our study since NIDDM represents the majority of the diabetic population. Reports in other models such as high fructose diet fed rats showed elevated responses with various vasoconstrictor agonists (Iyer and Katowich 1996; Song et al. 1997). Overfeeding of high caloric diets which contain lard causes increased sympathetic activity (Kaufman et al. 1991; Young et al. 1982). In male Beagle-Harrier dogs, 7 weeks of hyperlipidic hypercaloric diet produced abdominal obesity, increased diastolic and mean blood pressure (Verwaerde et al. 1996). Norepinephrine turnover is increased in heart, pancreas, interscapular brown adipose tissue and urinary norepinephrine excretion is also increased (Schwartz et al. 1983). α_1-adrenoceptors, which are situated in the periphery, are responsible for contraction of vascular and on non-vascular smooth muscle. α_1-Adrenoceptors are coupled through the Gq/11 mechanism and the agonists of these receptors phosphorylate phosphatidyl inositol to produce inositol triphosphate (IP_3), and diacylglycerol (DAG). IP_3 acts to release calcium from intracellular stores in the sarcoplasmic reticulum. DAG synergises with calcium to activate protein kinase C (PKC) which phosphorylates specific target proteins in the cell to change their function (Guimaraes and Moura 2001). Long-term α_1-adrenergic blockade strongly attenuates several of the fasting and postprandial

alterations in plasma variables of lipid and glucose metabolism induced by an extremely lipogenic diet (Fajardo and Deshaies 1998). So, the evaluation of the α-adrenoceptor role in HFD fed rat thoracic aorta will provide a quantitative relationship to hypertension and this will provide an avenue for further exploratory studies. It is already reported that 5-hydroxytryptamine (5-HT) and angiotensin II (Ang II) responses are altered in the aortic rings of STZ induced diabetic rats (Cinar et al. 2001; Orie and Aloamaka 1993) and high fructose diet fed rats (Iyer and Katowich 1996).

Few researchers have specifically investigated the altered vascular relaxation, and little information is available concerning the enhanced contractile responses to various agonists in HFD fed rats (Hodgkin 1991). In the present study we have shown altered biochemical parameters, enhanced vascular responses, altered sensitivity of α-adrenoceptors and receptor occupancy and response in HFD fed rat thoracic aorta. The purpose of these experiments was to address the hypothesis that high fat diet feeding causes an alteration in α-adrenoceptor mediated contractions in rat thoracic aortae.

2. Investigations and results

2.1. Body weights, abdominal and epididymal fat weights measurement

Rats fed with HFD gained more body weight, abdominal and epididymal fat weight than NPD fed rats after four weeks of feeding (Table 1).

After four weeks of HFD feeding the rats showed hyperglycemia, hypercholesterolemia and hypertriglyceridemia (Table 2). Fig. 1 shows the results of IPGTT carried out after 4 weeks of HFD feeding. There was a significant difference in the fasting glucose level between NPD fed and HFD fed rats. Plasma glucose levels were elevated in HFD fed rats when compared to NPD fed rats at all time points, but there was no statistically significant difference at the 15 min time interval.

2.2. Vascular responses

Helical strips of thoracic aorta were mounted in isolated organ baths for measurement of isotonic contractile forces. Cumulative CRCs were constructed in deliberately endothelium-denuded preparations obtained from both NPD fed and HFD fed rats. The cumulative CRCs of HFD fed rat thoracic aorta to PE (Fig. 2) shifted to the left and exhibited an elevated maximal response with no change in pD_2 (Table 3). Cumulative CRCs to KCl showed elevated responses in HFD fed rats when compared to NPD fed rats (Fig. 3). There was a significant change in E_{max} values (Table 3). The order of potency of agonists in both NPD and HFD fed rats was PE > KCl.

Table 1: General parameters in age matched NPD fed and HFD fed rats

Parameter	NPD fed	HFD fed
Body weight (g)	259.2 ± 5.3	315.0 ± 6.5 ***
Abdominal fat (g)	1.3 ± 0.2	2.9 ± 0.4*
Epididymal fat (g)	2.0 ± 0.1	3.3 ± 0.3*
Systolic BP	119.4 ± 0.9	141.0 ± 0.9**

All the measurements were made after four weeks of HFD feeding.
Each point is represented as mean ± S.E.M., n = 8.
* p < 0.05, ** p < 0.01, *** p < 0.001Vs NPD fed group

Table 2: Bio-chemical parameters in age matched NPD fed and HFD fed rats

Bio-chemical Parameter	NPD fed	HFD fed
Plasma glucose (mg/dl)	87.5 ± 2.5	111.0 ± 0.9***
Plasma cholesterol (mg/dl)	48.9 ± 3.7	87.5 ± 2.6***
Plasma triglycerides (mg/dl)	41.3 ± 1.7	89.7 ± 7.2***

All the measurements were made after four weeks of HFD feeding.
Each point is represented as mean ± S.E.M., n = 8.
*** p < 0.001 Vs NPD fed group

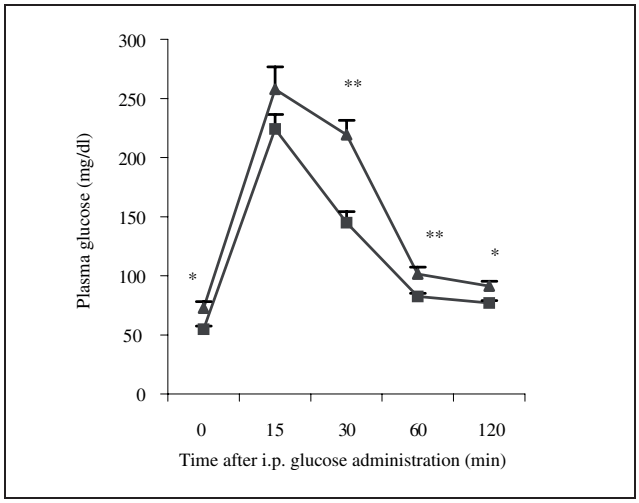

Fig. 1: Effect of HFD on intra peritoneal glucose tolerance test in rats, as compared to NPD fed group. All values are expressed as mean ± S.E.M., (n = 8)
* p < 0.05, ** p < 0.01, *** p < 0.005 Vs NPD fed group; –■– NPD fed, –▲– NPD fed

2.4. Systolic blood pressure recording

There was no significant difference in systolic blood pressure between NPD and HFD fed groups in the beginning of study (data was not shown). However, after 4 weeks of dietary manipulation, the rats fed HFD showed a signifi-

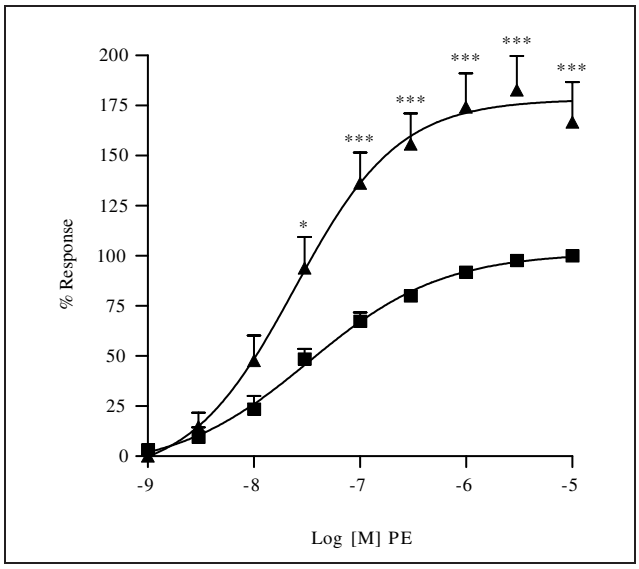

Fig. 2: Cumulative CRCs to PE in helically cut aortic strip preparations obtained from NPD fed and HFD fed rats. Each point is represented as mean ± S.E.M., (n = 9)
* p < 0.05, *** p < 0.001Vs NPD fed group; ■ NPD fed, ▲ HFD fed

Table 3: pD_2, E_{max} values (mm) for helically cut endothelial denuded aortic strips in response to PE induced contractions from the NPD fed and HFD fed rats at the end of four weeks of HFD feeding

	pD_2	E_{max}
NPD fed	7.5 ± 0.1	26 ± 3.0
HFD fed	7.6 ± 0.2	48 ± 3.0***

All the measurements were made after four weeks of HFD feeding.
Each point is represented as mean ± S.E.M., n = 9.
* $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$Vs NPD fed group

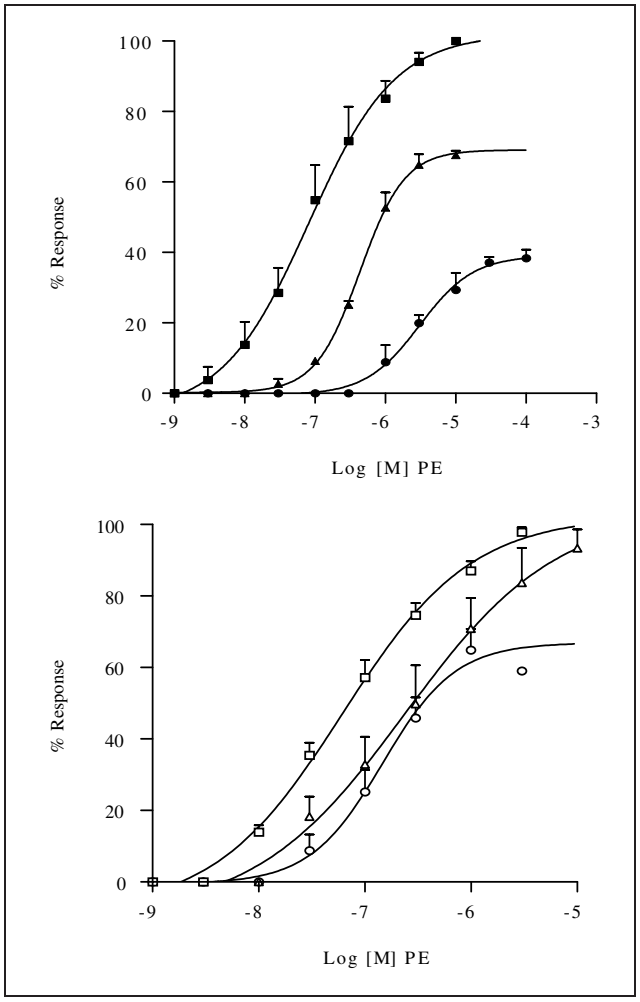

Fig. 3: Cumulative CRCs to PE in helically cut aortic strip preparations of NPD fed (top panel) and HFD fed rats (bottom panel) on treatment with irreversible antagonist phenoxybenzamine (PBZ). Each point is represented as mean ± S.E.M., (n = 3–6); ■ NPD fed, ▲ PBZ 1×10^{-8} M, ● PBZ 3×10^{-8} M; □ HFD fed, △ PBZ 1×10^{-8} M, ○ PBZ 3×10^{-8} M

cant ($p < 0.01$) increase compared to the NPD fed rats (Table 1).

2.5. Affinity of PE in NPD fed and HFD fed animals

By the Furchgott and Bursztyn method (Furchgott and Bursztyn 1967), K_A values were determined with PBZ. Two different concentrations of PBZ i.e. 10^{-8}, $3 \cdot 10^{-8}$ were used to determine K_A (Fig. 3). The double reciprocal plot i.e. 1/[A′] vs 1/[A] was made to get a straight line and the slope and intercept were determined. K_A value for NPD fed rats was 2.0 ± 0.4 μM (graph was not shown) and for HFD fed rats was 0.3 ± 0.1 μM (Fig. 4).

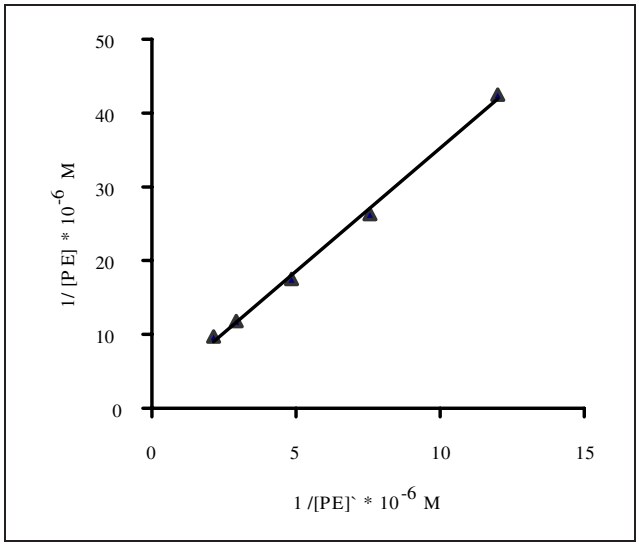

Fig. 4: Double reciprocal regression line plot of HFD fed rats

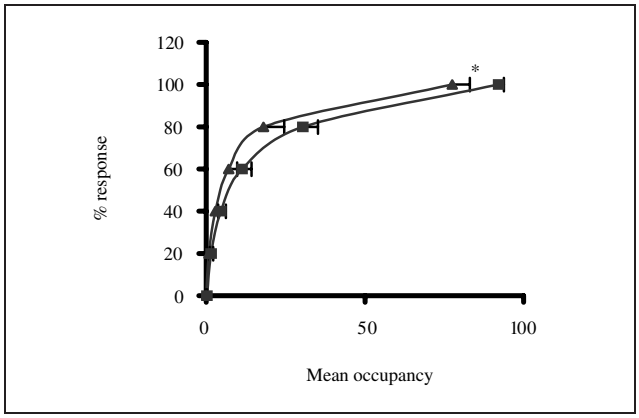

Fig. 5: Occupancy response relationship of PE in linear scale. Each point is represented as mean ± S.E.M., (n = 5)
* $p < 0.05$ Vs NPD fed group; –▲– HFD fed, –■– NPD fed

2.6. Relationship between receptor occupancy and response

Mean occupancy of the receptors was calculated by the method of Stephenson (1956). Mean occupancy of the receptors was plotted against concentration of PE and the curve was found to be rectangular hyperbolic in nature (Fig. 5). It was observed from these curves that PE needs to occupy $92.1 \pm 1.7\%$ of the receptor to produce maximal response. For 50% response, only $8.5 \pm 2.5\%$ of the total receptor pool needs to be occupied. Therefore, PE has $7.9 \pm 1.7\%$ of the spare receptors in NPD fed rats. For the half maximal response it was found to have $91.5 \pm 2.5\%$ of total receptor reserve. In the HFD fed rat thoracic aorta, the occupancy curve shifted slightly towards the left. In this case PE needed to occupy $77.5 \pm 5.6\%$ of receptors to produce its maximal response and $3.2 \pm 0.3\%$ to produce half-maximal response. PE thus has $22.5 \pm 5.6\%$ of spare receptors in the HFD fed rat thoracic aorta for the maximal response.

3. Discussion

It is clearly evident from the literature that HFD feeding in various animals such as dogs and rats (Storlien et al. 1986; Verwaerde et al. 1996) causes obesity. Obesity,

which is due to higher energy intake and reduced energy expenditure, leads to the development of NIDDM. The results from our studies have shown that HFD causes an increase in body-weight when compared to NPD fed after 4 weeks of feeding. Our results are similar to other reports in terms of body-weight and biochemical parameters and thus HFD feeding leads to insulin resistance (Khoursheed et al. 1995; Shibata et al. 1999). Hyperglycemia is observed in insulin resistant stages where glucose utilization is reduced. This work clearly shows elevated levels of plasma glucose, which indirectly suggests that HFD feeding causes insulin resistance. Oversupply of lipids causes insulin resistance (Kim et al. 1996). The excess lipid stores are preferred to glucose, according to the glucose fatty acid cycle (Randle et al. 1963). Plasma triglycerides and cholesterol are elevated in HFD fed rats (Shibata et al. 1999), which are the hallmarks of insulin resistance. Our findings from different biochemical estimations and IPGTT show that HFD causes a state similar to insulin resistance; estimation of plasma insulin reiterates these results. We found increased weights of fat stores at abdominal and epididymal regions. A significant correlation was observed between adipose tissue and systolic blood pressure elevation in Otsuka Long-Evans Tokushima fatty rats (Nagao et al. 2003). The present vascular studies demonstrate that the magnitude of responses to PE is significantly enhanced in HFD fed animals without corresponding changes in pD_2 values. Endothelial denudation obviates the mechanisms such as impairment of NO release, increased destruction of NO and substrate availability for the production of NO. The enhanced responses to various agonists may be receptor mediated or non-receptor mediated. The role of non-receptor mediated mechanisms in the enhanced contraction to contractile agents is eliminated by the absence of alteration in response to KCl. Where KCl mediated contractile responses in vascular smooth muscle cells are due to an influx of Ca^{2+} via voltage dependent Ca^{2+} channels. Enhanced contractile responses might be due to alteration of α-adrenoceptors. After four weeks of HFD feeding systolic blood pressure was elevated. This can be due to elevated vascular contractility caused by various agonists.

We hypothesize that this enhanced contractility might be due to alteration of α-adrenoceptors. The mean pD_2 values for PE were 7.5 ± 0.1 and 7.6 ± 0.2 in the NPD fed rat and HFD fed rat thoracic aorta, respectively. This suggests that there is no alteration in the sensitivity to PE in hypertension by HFD feeding.

K_A value and concentration-occupancy relationship determinations are powerful tools to identify the changes in receptor characteristics at the functional level. Furchgott's analysis represents the best known method for the evaluation of the K_A of an agonist in functional pharmacology. The most interesting finding of the present study was that there was a significant increase in affinity for PE to α-adrenoceptors of the aorta from HFD fed compared to NPD fed rats. The K_A value of PE is decreased almost six-fold in HFD fed rats when compared to NPD fed animals. The concentration-occupancy curve clearly shows a leftward shift in receptor occupancy in HFD fed rats compared to NPD fed rats and increased α-adrenoceptor reserve was observed in HFD fed animals. Doggrell (1992) also reported the same concentration-occupancy curve values for NPD fed rat thoracic aortae. Further, this increased contractility could be due to an increased receptor concentration within the cell membrane or to an increased intrinsic efficacy. Radioligand binding studies provide a useful method to differentiate these mechanisms. Further studies on the second messenger systems and endothelial intact preparations will provide valuable insights into the mechanisms involved in vascular reactivity alteration. The results strongly suggest that hypertension in HFD fed rats is associated with altered α-adrenoceptor status in hypertension.

4. Experimental

The institutional animal ethics committee of the National Institute of Pharmaceutical Education and Research (NIPER) approved all animal studies. Male Sprague-Dawley rats were procured from NIPER's central animal facility, weight range of 160–200 grams, and kept at controlled environmental conditions with room temperature 22 ± 2 °C and 12-h light/dark cycles. All the animals had free access to food and water. The rats were divided into two dietary groups and fed with standard rat normal pellet diet (NPD) (3.8 kcal/g, carbohydrate 67%, protein 21%, fat 12% kcal) and high fat diet (5.3 kcal/g, carbohydrate 17%, protein 25%, fat 58% kcal). Composition of HFD (100 g) was as follows: Powdered pellet diet 364 g, lard 310 g, casein 250 g, cholesterol 10 g, DL-methionine 3 g, yee-sac powder 1 g, vitamin and mineral mix powder 60 g, sodium chloride 2 g.

4.1. Biochemical measurements

Blood samples were collected from the retro orbital plexus in anaesthetized rats (pentobarbitone 45 mg/kg, i.p.,) and immediately centrifuged (Remi, India) at 5000 rpm for the separation of plasma. Plasma was stored at −10 °C until assayed. The plasma was used for the estimation of glucose (Qualigens, Mumbai, India), triglycerides, and cholesterol (Chema diagnostics, Italy) by commercial kits.

4.2. Intra peritoneal glucose tolerance test (IPGTT)

Glucose tolerance tests were carried out after four weeks of feeding of both NPD and HFD. After an overnight fast, blood samples were collected from the retroorbital plexus. Glucose levels were measured at time zero (0 min) and glucose was injected into the rats (2 g/kg/4 ml, i.p.,). Additional blood samples were taken at 15, 30, 60 and 120 min following the glucose load. Plasma glucose levels were measured by the glucose oxidase reaction (GOD/POD) in a commercial kit (Qualigens, Mumbai, India).

4.3. Systolic blood pressure recording

Systolic blood pressure (BP) was assessed by the tail cuff method (IITC Inc, Lifescience Instruments, CA, USA) under conscious conditions at the beginning and at 4-week dietary manipulations. The average of three pressure readings was recorded for each measurement.

4.4. Vascular studies

After 4 weeks of HFD feeding, rats were sacrificed by cervical dislocation and the thoracic aorta was isolated from the heart to the diaphragm. It was freed from fat and connective tissue. Care was taken not to stretch the vessel. With the help of a steel rod, endothelium was deliberately denuded. Helical strips of aorta of 3 mm in width and 20 mm in length (Kamata and Makino 1997) were made by cutting it spirally with sharp iris scissors and placed in a 10 ml organ bath (Inco, India) containing a modified Krebs Henseleit solution (KHS) (in mM: NaCl – 118, KCl – 4.7, KH_2PO_4 – 1.2, $Mg\,Cl_2 \cdot 6\,H_2O$ – 1.2, $CaCl_2 \cdot 2\,H_2O$ – 2.6, $NaHCO_3$ – 25, and glucose – 11.1) of pH 7.4 (Shastri et al. 2001). The solution was continuously aerated with carbogen (95% O_2 + 5% CO_2) at 37 °C. Two thoracic aortic strips were prepared from each rat. One end of the strip was tied to a tissue holder and the other end to an isotonic transducer. Tissues were equilibrated for 1 h under resting tension of 2 g. Bath fluid was changed every 15 min. At the beginning of each experiment, aortic strips were depolarized with 30, 60, and 120 mM KCl and responses were recorded isotonically on a physiograph (Bio Devices, Ambala, India) to check the contractility of vessels. Strips were then thoroughly washed with KHS buffer and allowed to equilibrate. Vascular responses to PE were recorded. After excision of the thoracic aorta, the epididymal and abdominal fat was removed and weighed.

4.5. Analysis of concentration response curves (CRC)

The data were quantified by determining both the maximal effect (E_{max}) and the concentration of the agonist necessary to produce 50% of its own response (EC_{50}). The EC_{50} values were converted to negative logarithms and expressed as pD_2 values.

4.6. Estimation of affinity of agonist (K_A)

The affinity of agonists was measured by the Furchgott and Bursztyn method (Furchgott and Bursztyn 1967). In this method the tissue was treat-

ed with non-equilibrium or irreversible antagonist phenoxybenzamine (PBZ) for 15 min. The unreacted PBZ was washed out with a continuous slow passage of 500 ml KHS over a period of 15 min. The concentration of the agonist that produced the equivalent responses before and after PBZ treatment was considered as [A] and [A′] respectively. Then a double reciprocal plot i.e. 1/[A′] vs 1/[A] was made to get a straight line and the slope and intercept were determined. From this, the K_A value was calculated using the following equation:

$$\frac{1}{[A]} = \frac{1}{q[A']} + \frac{1-q}{qK_A} \quad (1)$$

where [A] is the concentration of agonist necessary to give a fixed percentage of the maximal response before PBZ treatment, [A′] is the concentration of agonist necessary to give the same percentage of maximal response after PBZ treatment, K_A is the equilibrium dissociation constant of agonist, and q is the fraction of initial receptor concentration remaining active (functional or non-alkylated) after PBZ treatment. The K_A value and the fraction of active receptors remaining (q) were calculated from slope and intercept of the straight line fitted by linear regression on the basis of the equation in which K_A equals (slope-1)/intercept and q equals 1/slope.

4.7. Determination of the relationship between receptor occupancy and response

There is a good evidence of a non-linear relationship between the receptor occupancy and response in many isolated tissues (Stephenson 1956). It has been suggested that irreversible inactivation of the receptor pool can lead to dextral displacement of the concentration response curve. From the knowledge of concentration required producing a particular response and K_A value, the agonist's percentage occupancy of the receptors was calculated by the following formula, and plotted against the concentration with response (Thiyagarajan et al. 2002)

$$\% \frac{[RA]}{[RT]} = \frac{[A]}{[A] + K_A} \times 100 \quad (2)$$

where [RA] is the concentration of the receptor agonist complex, [RT] is the total receptor concentration and K_A is the dissociation constant of the agonist.

4.8. Drug solutions

The following drugs were used in this study: PE (Sigma Chemical Company, USA), phenoxybenzamine (Smith Kline & French Labs., Switzerland). All drugs were prepared according to their own solubilities and kept at $-10\,^{\circ}C$ as stock solutions in concentrations of 10^{-2} M. They were diluted on the day of experimentation. Drug concentrations are reported as final molar concentration in the organ bath.

4.9. Statistical analysis

All results were expressed as mean ± S.E.M., Statistical differences between two means ($p < 0.05$) were determined by students t-test by using statistical computer software (GraphPad Prism 3.01).

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