Br. J. Pharmac. (1986), **87**, 157–165

Hypersensitivity to methoxamine in atria isolated from streptozotocin-induced diabetic rats

Liliana Canga & Leonor Sterin-Borda[1]

CEFAPRIN, CONICET, Serrano 665/69, (1414) Buenos Aires, Argentina

1 The reactivity to methoxamine (Met) of atria isolated from the hearts of normal and from acutely streptozotocin-diabetic rats has been studied.

2 Met (1×10^{-6}M) increased the tension of both normal and diabetic atria, but in diabetic atria, the dose-response curve to Met was shifted to the left and the efficacy of Met was enhanced.

3 Inhibitors of α-adrenoceptors blocked, in a competitive manner, the positive inotropic effect induced by Met in both types of atrial preparations.

4 Inhibitors of the cyclo-oxygenase pathway for arachidonic acid metabolism blocked the atrial response to Met in non-diabetic as well as in diabetic atria. The inhibition of prostacyclin synthetase prevented the effect of Met in normal atria, while blockers of thromboxane generation inhibited it in diabetic ones.

5 Agents that inhibit the activity of lipoxygenase(s) significantly reduced the positive inotropic action induced by Met in diabetic atria but failed to modify it in non-diabetic preparations.

6 These results show that diabetic atria are more sensitive to Met than normal atria. In diabetes the response to α-adrenoceptor stimulation could be mediated by oxidative products generated via thromboxane synthetase and lipoxygenase(s) activities; whereas in normal preparations the action of Met may involve the release of prostacyclin.

Introduction

Recent studies employing animal models have shown cardiac abnormalities associated with diabetes mellitus (Melhotra *et al.*, 1981; Fein *et al.*, 1981) and significant alterations at the biochemical level may be responsible for some aspects of diabetic cardiomyopathy (Malhotra *et al.*, 1981; Pierce & Dhalla, 1981). Thus, changes in sensitivity to α-adrenoceptor agonists (noradrenaline and methoxamine) have been detected in tissues isolated from diabetic rats. Ramanadham *et al.* (1984) reported not only increased sensitivity of tail artery strips but also a decreased response to α-agonists in thoracic aorta isolated from diabetic rats. However, alterations in cardiac function can develop without coronary vascular complications (Penpargkul *et al.*, 1980; Dillmann, 1980; Vadlamudi *et al.*, 1982). Recently, attention has been focused on the changes which occur in isolated myocardium from rats with chemically-induced diabetes of short duration. The studies indicate that the responsiveness of the diabetic myocardium to adrenoceptor agonists is altered (Foy & Lucas, 1975; 1978).

The presence of α-adrenoceptors in adult rat atrial strip preparations have been suggested by Nakashima *et al.* (1973), but little is known about myocardial reactivity to α-agonists after short-term streptozotocin treatment. There is evidence, however, that one or more metabolites of arachidonic acid could be implicated in the pathophysiological alterations observed in diabetes mellitus (Metz, 1981; Chen & Robertson, 1979). Arachidonic acid (AA), a fatty acid bound to membrane phospholipids, is the precursor of derivatives that regulate cardiac function. AA is released by the stimulation of specific cell-surface receptors and subsequent activation of phospholipases of the A_2-type (Lapetina, 1982). The AA is metabolized by two enzymes systems (cyclo-oxygenase and lipoxygenase(s)) forming prostaglandins, thromboxanes, hydroxyeicosatetraenoic acids, leukotrienes and other compounds (Moncada & Vane, 1978). In a recent study, we have examined the contractile response to exogenous AA of atria from normal and diabetic rats and have established that, during diabetes, the metabolism of AA is shifted towards thromboxane B_2 (TXB_2) formation while in normal atria it is directed to prostacyclin (PGI_2) production (Canga *et al.*, 1984).

[1]Author for correspondence.

In view of these precedents, the present study was undertaken to explore the inotropic responses to α-adrenoceptor stimulation, induced by methoxamine (Met), of atria from normal and streptozotocin-diabetic rats. Results show that although a positive inotropic effect is induced in both groups of atria when α-adrenoceptors are stimulated by Met; atria from diabetic rats are more sensitive to α-adrenoceptor activation than those from normal rats. The mechanism appears to be that Met, upon binding to the myocardial α-adrenoceptor, triggers the generation of oxidative products of endogenous AA and they, in turn, enhance the positive inotropic effect of Met. In diabetic atria lipoxygenase(s) metabolites and thromboxanes are the effectors, whereas, in non-diabetic atria, α-adrenoceptor stimulation is related to metabolites generated via prostacyclin synthetase.

Methods

Isolated atria preparations of the rat

Wistar male rats, weighing between 200–250 g, were used. The animals were killed by decapitation with a guillotine. The entire heart was quickly removed and placed in Petri dishes filled with a modified Krebs-Ringer-Bicarbinate (KRB) solution of the following composition (mM): Na^+ 145, K^+ 6.02, Ca^{2+} 1.22, Mg^{2+} 1.33, Cl^- 126, HCO_3^- 25.3, SO_4^{2-} 1.33, PO_4^{2-} 1.20 and glucose 5.5.

Atria were separated from ventricles, carefully dissected, attached to a glass holder and immersed in a tissue chamber filled with 20 ml of KRB solution. This tissue bath medium was gassed with 95% O_2 and 5% CO_2 and kept at a constant temperature (30°C) and pH (7.4) throughout the experiments. One end of the preparation was anchored to the glass holder and the other was connected to a force transducer (Statham UC3-Gold Cell) coupled to an ink-writing oscillograph (San Ei 180). A constant resting tension of 750 mg was applied to the auricles by means of a micrometric device and the activity of spontaneously beating preparations was analysed in terms of isometric contractile tension. This variable, taken from the oscillographic records, is referred to in the text as the isometric developed tension (I.D.T.) and expressed in mg. The atria were allowed to equilibrate for 60 min. The magnitude of the contractile tension recorded at this moment was considered as the initial control tension before administering drugs. This initial (control) tension was taken as 100%, and the variations induced by drugs were expressed as % changes compared to the control tension. The frequency of contractions, measured in beats per min, was not modified before the addition of the drugs (Table 2). Cumulative dose-response curves for Met were made according to the method described by Van Rossum (1963). Single doses were administered in volumes of 0.01 to 0.025 ml of KRB solution. The total volume added to the bath never exceeded 0.1 ml. The time interval between doses was that required by each one to produce a maximal effect (10 min).

Induction of diabetes

Experimental short-term diabetes was induced in male Wistar rats (200–250 g) with a single i.p. dose of streptozotocin (100 mg kg^{-1}). Streptozotocin was dissolved in citrate buffer (pH 4.8) prior to the injection. A colorimetric enzymatic method (glycaemia enzymatic) for 'true glucose' determination in blood, was used. Animals with plasma glucose above 300 mg dl^{-1} and with ketonuria were considered to be diabetic. The rats were killed 72 h after streptozotocin injection. Blood samples were taken without anaesthesia by intramyocardial puncture before killing the animal.

Drugs

Acetylsalicylic acid (ASA), nordihydroguaiaretic acid (NDGA), imidazole, dithizone and streptozotocin were purchased from Sigma Chemical Co.; indomethacin from Merck, Sharp & Dohme; phenoxybenzamine (Pbz) and tranylcipromine from Smith, Kline & French; L-8027 (Nictindol) from Labaz Lab.; methoxamine (Met) from Burroughs Wellcome Co.; phentolamine from Ciba; prazosin from Pfizer Lab. and glycaemia enzymatic from Wiener Lab. Stock solutions of acetylsalicylic acid, imidazole, dithizone, indomethacin, tranylcipromine, Met, phentolamine and prazosin were dissolved in distilled water whereas NDGA and L-8027 were dissolved in dimethylsulphoxide (DMSO), as previously described (Sterin-Borda *et al.*, 1983). The drugs were diluted in the bath to achieve the final concentrations shown in Table 2. At these dilutions the solvent itself had no effect. Rat isolated atria were incubated for 30 min with α-adrenoceptor antagonists and/or inhibitors of cyclo-oxygenase or lipoxygenase(s) before adding increasing amounts of Met.

Statistics

Experimental results were compared by means of Student's *t* test, following the tables given by Fisher & Yates (1957). Differences between means were considered significant if $P2 = 0.05$ or less.

Results

Dose-response curves to methoxamine in atria isolated from normal and diabetic rats and the effect of α-adrenoceptor antagonists

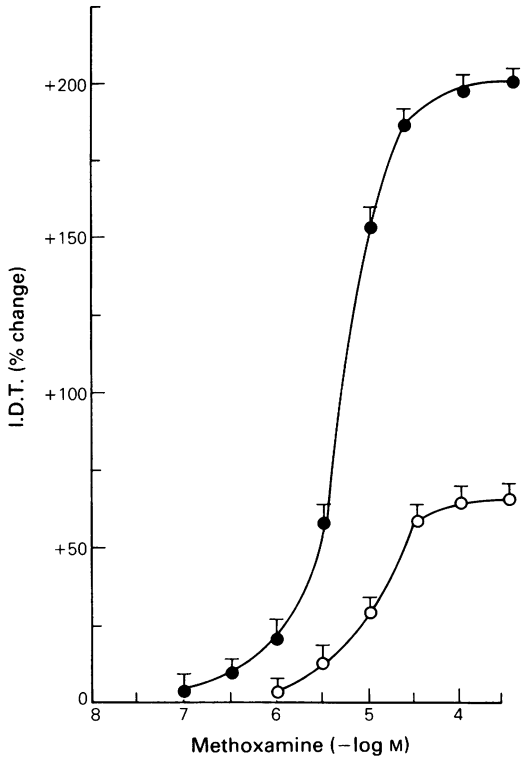

Figure 1 Cumulative dose-response curves to methoxamine in atria isolated from normal (○) and diabetic (●) rats. I.D.T., isometric developed tension (dF/dt), is expressed as % change vs. initial controls. Each point represents the mean, and vertical lines s.e.mean, of 8 experiments.

Figure 1 shows the effect of increasing concentrations of methoxamine (Met) on the contractile tension of spontaneously beating atria obtained from normal and diabetic rats. It can be seen that Met induced a concentration-dependent increase in tension in both groups of atria. Both the efficacy and potency were greater on atria from diabetic rats (E_{max}: 180; K_D: 1.02×10^{-5} M) than those from normal, control, rats (E_{max}: 78; K_D: 2.0×10^{-5} M).

To assess the role of α-adrenoceptors in the contractile effect of Met, both groups of atria were incubated with α-adrenoceptor antagonists such as phentolamine, prazosin or phenoxybenzamine before the addition of Met. Results shown in Figure 2 and Table 1 indicate that in the presence of these agents, the positive inotropic action of Met was antagonized in a competitive manner both in normal atria in those from diabetic rats. The isometric tension developed after the addition of the α-antagonists both in normal and in diabetic atria had a similar magnitude (Table 2).

Effects of inhibitors of cyclo-oxygenase and lipoxygenase(s) activities on the contractile action of methoxamine

Several inhibitors acting on different pathways that could result in stimulation of atrial tension, were used in order to determine the nature of the mechanism triggering the hypersensitivity to Met in diabetic atria. Figure 3 illustrates the effect of two blockers of cyclo-oxygenase activity. It can be seen that in the presence of acetylsalicylic acid (1.8×10^{-4} M) or indomethacin (1.2×10^{-6} M), the positive inotropic action of Met was significantly reduced in auricles from both normal and diabetic rats.

In order to elucidate which of the prostanoids could be involved in this effect, auricles were incubated with known inhibitors of prostacyclin synthetase (tranylcipromine) and of thromboxane synthetase (L-8027 and imidazole) activities. As can be seen Figure 4, in the presence of tranylcipromine (2.5×10^{-4} M) the stimulant effect of Met was significantly diminished in both normal and diabetic auricles although the decrease was more marked in normal atria. On the other hand, inhibition of thromboxane synthetase by imidazole (1×10^{-3} M) or by L-8027 (1×10^{-6} M) reduced the positive inotropic effect of Met only in diabetic atria and failed to do so in normal preparations. The modification of the action of Met by inhibitors of lipoxygenase(s) activity (i.e. NDGA or dithizone) is shown in Figure 5. Although in normal atria the increment in contractile tension evoked by Met was not altered by NDGA (1×10^{-5} M) or by dithizone (1×10^{-5} M), a significant inhibition of the positive inotropic effect of Met was observed in diabetic atria previously incubated with any of these two agents.

Discussion

The present study was performed in order to investigate the influence of methoxamine (Met) on the contractile tension of atria isolated from normal and acutely-diabetic rats. Our results demonstrate that Met is able to induce a dose-dependent increase of contractile tension in both types of atrial preparations. The inotropic action of Met was not accompanied by changes in the rate of contraction. The positive inotropic effect elicited by stimulation of α-adrenoceptors has also been described in isolated myocardium of various mammalian species (Nakashima *et al.*, 1973; Wagner & Brodde, 1978; Schümann *et al.*, 1978; Benfey, 1973). In addition, we found that auricles isolated from diabetic rats showed an increased sensitivity to Met compared to that of non-diabetic controls (Figure 1). In turn, in the presence of α-adrenoceptor blocking agents (i.e. phentolamine,

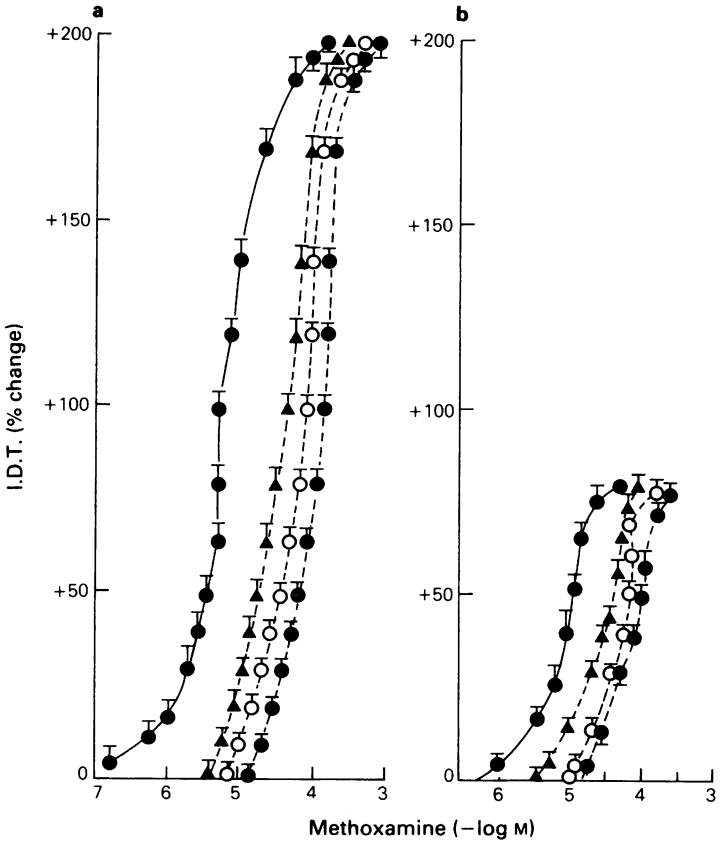

Figure 2 Inotropic effect of methoxamine (●——●) in normal (b) and diabetic (a) atria. Influence of phenoxybenzamine (● - - - ●), phentolamine (○ - - - ○) and prazosin (▲ - - - ▲). Each point represents the mean, and vertical lines s.e.mean, of 6 experiments. Other details as in Figure 1.

Table 1 Influence of α-adrenoceptor antagonists on the positive inotropic effect of methoxamine in normal and diabetic atria

	Normal		*Diabetic*	
	E_{max}	K_D	E_{max}	K_D
Methoxamine	78 ± 6	2.0×10^{-5}M	180 ± 12	1.08×10^{-5}M
Phentolamine (10^{-8}M)	77 ± 5	7.0×10^{-5}M	189 ± 10	8.47×10^{-5}M
Phenoxybenzamine (10^{-8}M)	72 ± 3	3.0×10^{-5}M	201 ± 15	12.70×10^{-5}M
Prazosoin (10^{-8}M)	65 ± 4	6.3×10^{-5}M	195 ± 14	4.10×10^{-5}M

Values are ± s.e.mean of 6 experiments in each group. Differences are significant ($P < 0.01$). Thes values were obtained by fitting data of Figure 2 to Michaelis-Menten equation and taken to lineal relationship and constructed the equivalent to Lineweaver-Burk plot.

Table 2 Effect of different drugs on the spontaneous activity of rat isolated atria

	Normal atria		*Diabetic atria*		
Drugs	*Tension*	*Frequency of contractions*	*Tension*	*Frequency of contractions*	n
None	320 ± 8	140 ± 6	350 ± 15	140 ± 7	8
Phe (1×10^{-8}M)	315 ± 3	129 ± 10	335 ± 6	130 ± 10	4
Pbz (1×10^{-8}M)	315 ± 7	138 ± 10	320 ± 15	135 ± 12	4
Praz (1×10^{-8}M)	310 ± 8	140 ± 5	330 ± 12	139 ± 7	4
ASA (1.8×10^{-4}M)	310 ± 7	138 ± 6	330 ± 10	140 ± 6	5
Indo (1.2×10^{-6}M)	300 ± 10	136 ± 8	320 ± 6	138 ± 5	5
NDGA (1×10^{-5}M)	300 ± 12	129 ± 9	315 ± 8	130 ± 10	5
Dith (1×10^{-5}M)	315 ± 4	140 ± 7	325 ± 6	142 ± 5	5
Tranyl (2.5×10^{-4}M)	320 ± 10	130 ± 8	310 ± 10	135 ± 5	4
Imidazole (1×10^{-3}M)	300 ± 10	138 ± 9	345 ± 7	136 ± 6	6
L-8027 (1×10^{-6}M)	300 ± 9	137 ± 7	350 ± 8	136 ± 8	5

Values are mean ± s.e.mean; tension measured in mg and frequency of contractions measured in number of beats per min. The initial control values (no additions) were recorded after equilibration (60 min following setting up of the preparation) and, the experimental ones, at 30 min after administration of each drug (see Method). *n* = number of preparations. Phe: phentolamine; Pbz: phenoxybenzamine; Praz: prazosin; ASA: acetylsalicylic acid; Indo: indomethacin; NDGA: nordihydroguaiaretic acid; Dith: dithizone; Tranyl: tranylcipromine.

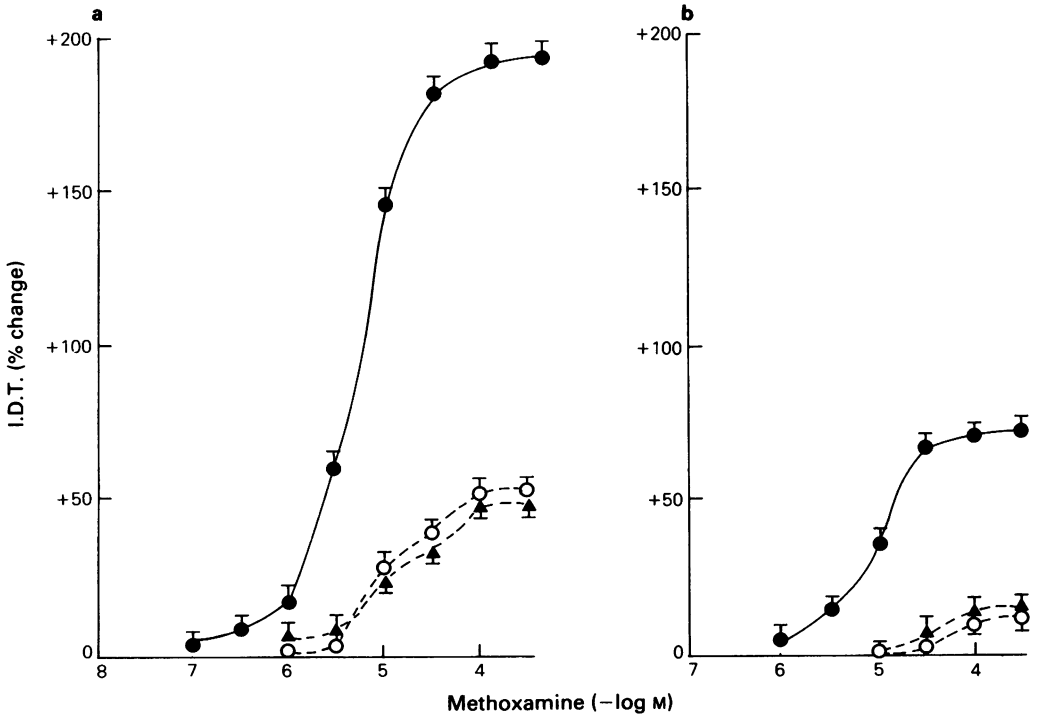

Figure 3 Inotropic effect of methoxamine (●) in normal (b) and diabetic (a) atria in the presence of cyclo-oxygenase inhibitors: acetylsalicylic acid (○) and indomethacin (▲). Each point represents the mean, and vertical lines s.e.mean, of 5 experiments. Other details as in Figure 1.

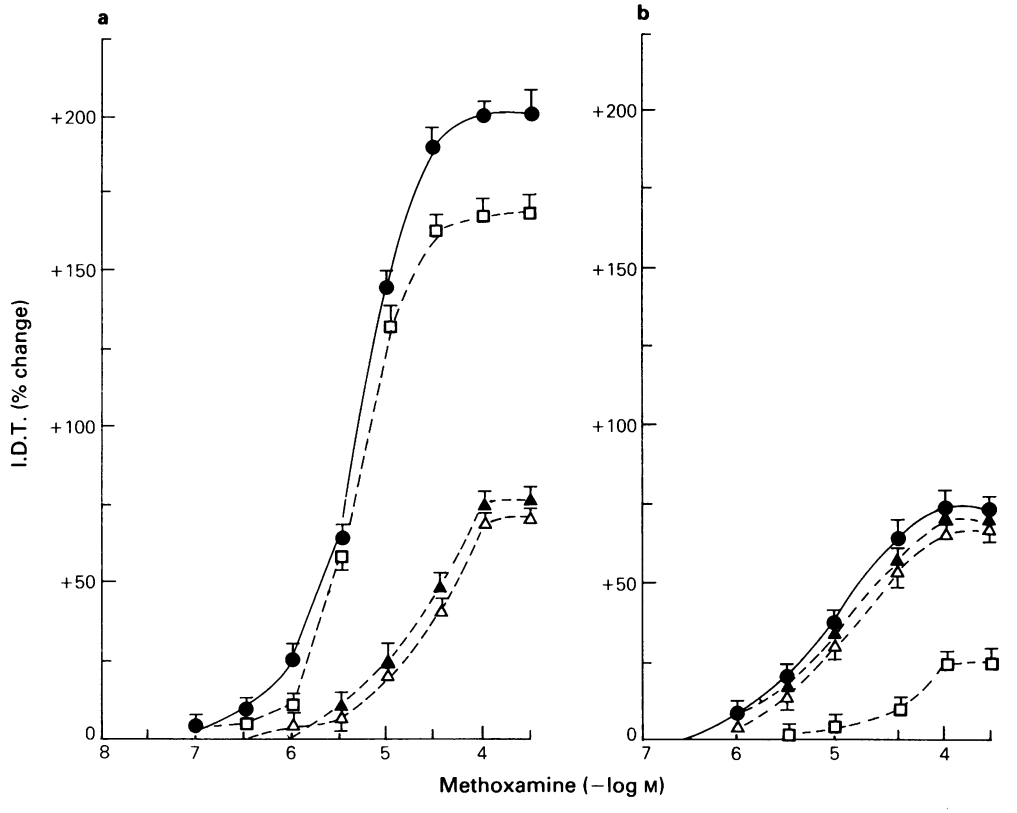

Figure 4 Influence of tranylcipromine (□), imidazole (▲) and L-8027 (△) on the inotropic effect of methoxamine (●) in isolated atria from normal (b) and diabetic (a) rats. Each point represents the mean, and vertical lines s.e.mean of 4 experiments. Other details as in Figure 1.

prazosin or phenoxybenzamine) the magnitude of the stimulant effect of the α-agonist was inhibited in a competitive manner in normal as well as in diabetic hearts (Figure 2 and Table 1). These findings are consistent with a previous report (Downing *et al.*, 1983), in which Met evoked positive inotropic responses in myocardium from normal and alloxan-induced diabetic lambs. Hyperresponsiveness to α-adrenoceptor stimulation was also found in diabetic lamb hearts (Downing *et al.*, 1983).

On the other hand, we also showed (Figure 3) that the increased response to Met in normal and in diabetic atria was abolished by indomethacin or acetylsalicylic acid, at concentrations known to inhibit cyclo-oxygenase activity (Vane, 1971). This supports the notion that in normal and in diabetic atrial tissue, some product derived from arachidonic acid (AA) metabolism via the cyclo-oxygenase pathway could be involved in the cardiac effect of Met. To elucidate which cyclo-oxygenase end-product could be participating in this event, both groups of atria were incubated with tranylcipromine, an inhibitor of prostacyclin synthetase (Gryglewski *et al.*, 1976), or with imidazole or L-8027, thromboxane synthetase blocking agents (Moncada *et al.*, 1977), before testing their responsiveness to Met. Tranylcipromine was more effective in blocking the response to Met in normal atria than in diabetic ones. In contrast, thromboxane synthetase blocking agents only diminished the stimulant effect to Met in diabetic atria (Figure 5). These findings suggest that under normal conditions, prostacyclin appears to be responsible for the positive inotropic action of Met, while in the diabetic state, thromboxanes could be mediating this effect. In previous studies from our laboratory, positive inotropic actions of prostacyclin (PGI_2) and thromboxane B_2 (TXB_2) in rat isolated atria have been found (Sterin-Borda *et al.*, 1979; 1980; 1981). Results obtained from radioconversion of labelled arachidonic acid by normal atria confirmed that 6-oxo-

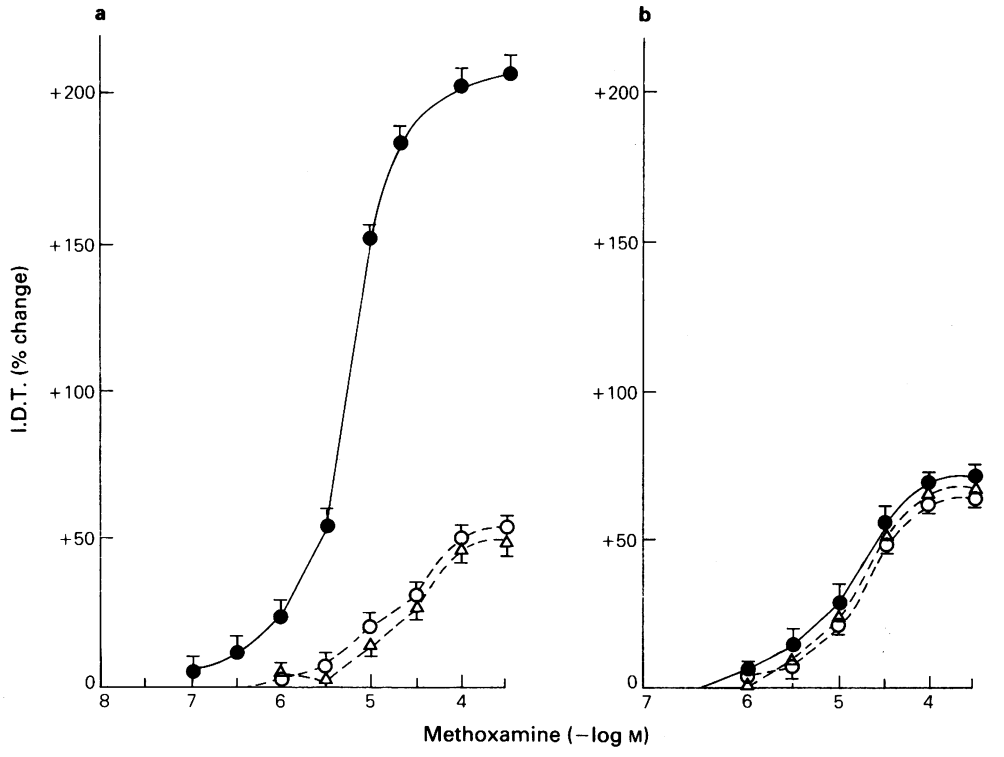

Figure 5 The positive inotropic effect of methoxamine (●) in normal (b) and dibetic (a) atria and the influence of lipoxygenase(s) inhibitors: nordihydroguaiaretic acid (○) and dithizone (△). Each point represents the mean, and vertical lines s.e.mean, of 5 experiments. Other details as in Figure 1.

prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$; the non-enzymatic metabolite of PGI_2) was the product of AA metabolism generated in preference to TXB_2. Moreover, the distribution of metabolites from exogenous AA was altered in diabetic atria; an increased production of TXB_2 with a decreased formation of 6-oxo-$PGF_{1\alpha}$ was observed (Canga *et al.*, 1984). The fact that tranylcipromine was able to block slightly the response to Met in diabetic atria suggests that PGI_2 is also generated in these preparations. An interaction between α-adrenoceptor-mediated stimulation by Met and prostacyclin release has been described by us in rat aorta (Borda *et al.*, 1983b) and in vas deferens (Borda *et al.*, 1983a). In normal atria the response to Met was not modified after incubation with NDGA or dithizone. Therefore, we propose that products of the lipoxygenase(s) pathway do not appear to be involved in the α-agonist-induced contractile tension (Figure 5). However, during diabetes a blocker of lipoxygenase(s) activity was required to diminish the stimulant influence of Met (Figure 4), suggesting the participation of some lipoxygenase(s)-derived product as an activating factor for the augmented thromboxane production which, in turn, is able to induce an increase in contractile tension. This is in keeping with a previous report which stated that some groups of compounds originating from AA by the action of lipoxygenase(s) produced a strong and long-lasting contraction of lung parenchymal strips, and this was proved to be mediated by the release of TXA_2 (unstable precursor of TXB_2) (Piper & Samhoun, 1981). Moreover, in coronary arteries from diabetic dogs, we showed that sodium arachidonate induced vasoconstriction via TXA_2 generated by lipoxygenase(s) catalyzed reactions (Sterin-Borda *et al.*, 1982). Because the stimulant action of Met was not completely abolished after blocking thromboxane synthetase activity we cannot rule out the possibility that the lipoxygenase(s) product can also *per se* elecit a positive inotropic effect. Until now the underlying mechanism of the positive inotropic effect mediated by α-adrenoceptors has been unclear. We suggest that the effects observed with Met could be explained by a mechanism which couples activation of cardiac α-adrenoceptors to chan-

ges in the metabolism of arachidonic acid. From our results we conclude that in normal atria the response to α-adrenoceptor stimulation induced by Met could be mediated through liberation of arachidonic acid which is predominantly metabolized via cyclo-oxygenase to produce prostacyclin which, in turn, may be responsible for the final biological response (i.e., the positive inotropic effect). However, in diabetic atria, supersensitivity to Met may be attributed to the diversion of AA metabolism towards an increased production of TXA_2 and a lipoxygenase(s)-derived product.

This was was supported by Grant 6638 from CONICET, Argentina. We gratefully acknowledge Mrs Elvita Vannucchi for her technical assistance.

References

BENFEY, B.G. (1973). Characterization of alpha adrenoceptors in the myocardium. *Br. J. Pharmac.*, **48**, 132–138.

BORDA, E.S., PEREDO, H. & GIMENO, M.F. (1983a). Alpha adrenergic stimulation modified prostaglandins release in vas deferens. *Prostaglandins*, **26**, 701–710.

BORDA, E.S., STERIN-BORDA, L., GIMENO, M.F., LAZZARI, M. & GIMENO, A.L. (1983b). Is prostacyclin subserving a vasodilator effect of methoxamine involving alpha adrenoceptors? *Experimentia*, **39**, 894–896.

CANGA, L., STERIN-BORDA, L., BORDA, E.S., PEREDO, H. & GIMENO, A.L. (1984). The positive inotropic effect of sodium arachidonate on auricles from diabetic rats. *Eur. J. Pharmac.*, **110**, 47–54.

CHEN, M. & ROBERTSON, R.P. (1979). Effects of prostaglandin synthesis inhibitors on human insulin secretion and carbohydrate tolerance. *Prostaglandins*, **18**, 557–567.

DILLMANN, W.H. (1980). Diabetes mellitus induces changes in cardiac myosin of the rat. *Diabetes*, **29**, 579–582.

DOWNING, S.E., LEE, J.C. & FRIPP, R.R. (1983). Enhanced sensitivity of diabetic hearts to α-adrenoceptor stimulation. *Am. J. Physiol.*, **245**, H808–H813.

FEIN, F.S., STROBECK, J.E., MALHOTRA, A., SCHEUER, J. & SONNENBLICK, E.H. (1981). Reversibility of diabetic cardiomyopathy with insulin in rats. *Circulation Res.*, **49**, 1251–1261.

FISHER, R.A. & YATES, F. (1957). *Statistical Tables for Biological, Agricultural and Medical Research.* 5th edition. New York: Hafner Publishing Co.

ROY, J.M. & LUCAS, P.D. (1976). Effect of experimental diabetes, food deprivation and genetic obesity on the sensitivity of pithed rats to autonomic agents. *Br. J. Pharmac.*, **57**, 229–234.

FOY, J.M. & LUCAS, P.D. (1978). Comparison between spontaneously beating atria from control and streptozotocin-diabetic rats. *J. Pharm. Pharmac.*, **30**, 558–562.

GRYGLEWSKI, R.J., BUNTING, S., MONCADA, S., FLOWER, R.J. & VANE, J.R. (1976). Arterial walls are produced against deposition of platelet thrombi by a substance (prostaglandin X) which they made from postaglandin endoperoxides. *Prostaglandins*, **12**, 685–714.

LAPETINA, E.G. (1982). Regulation of arachidonic acid production: role of phospholipases C and A_2. *Trends Pharmac. Sci.*, **3**, 115–118.

MALHOTRA, A., PENPARGKUL, S., FEIN, F.S., SONNENBLICK, E.H. & SCHUERER, J. (1981). The effect of streptozotocin-induced diabetes in rats on cardiac proteins. *Circulation Res.*, **49**, 1243–1250.

METZ, S.A. (1981). Feedback modulation of glucose-induced insulin secretion by arachidonic acid metabolites: possible molecular mechanisms and relevance to diabetes mellitus. *Prostaglandins & Medicine*, **7**, 581–589.

MONCADA, S., BUNTING, S., MULLANE, K.M., THROGOOD, P., VANE, J.R., RAZ, A. & NEEDLEMAN P. (1977). Imidazole: a selective potent antagonist of thromboxane synthetase. *Prostaglandins*, **13**, 611–618.

MONCADA, S. & VANE, J.R. (1978). Unstable metabolites of arachidonic acid and their role in haemostasis and thrombosis. *Br. med. Bull.*, **34**, 129–134.

NAKASHIMA, M., TSURU, H. & SHIEGEI, T. (1973). Stimulant action of methoxamine in the isolated atria of normal and 6-prophyl-2-thiouracil fed rats. *Jap. J. Pharmac.*, **23**, 307–312.

PENPARGKUL, S., SCHAIBLE, T., YIPINSTOI, T. & SCHEUER, J. (1980). The effect of diabetes on performance and metabolism of rat hearts. *Circulation Res.*, **47**, 911–921.

PIERCE, G.N. & DHALLA, N.S. (1981). Cardiac myofibrillar ATPase activity in diabetic rats. *J. mol. cell Cardiol.*, **13**, 1–7.

PIPER, P.J. & SAMHOUM, M.N. (1981). The mechanism of action of leukotrienes C_4 and D_4 in guinea-pig isolated perfused lung and parenchymal strips of guinea-pig, rabbit and rat. *P'rostaglandins*, **24**, 793–803.

RAMANADHAM, S., LYNESS, W.H. & TENNER, T.E. (1984). Alterations in aortic and tail artery reactivity to agonists after streptozotocin treatment. *Can. J. Physiol. Pharmac.*, **62**, 418–423.

SCHÜMANN, H.J., WAGNER, J., KNORR, A., REIDEMESTER, J., SADONY, V. & SCHRAMM, G. (1978). Demonstration in human atrial preparations of α-adrenoceptors mediating positive effects. *Naunyn-Schmiedebergs Arch. Pharmac.*, **302**, 333–336.

STERIN-BORDA, L., BORDA, E. DEL CASTILLO, E.J., GIMENO, M.F. & GIMENO, A.L. (1982). Mechanisms of coronary vasoconstriction induced by Na arachidonate in experimentally diabetic dogs. *Experientia*, **38**, 835–837.

STERIN-BORDA, L., BORDA, E., FINK, S. & BRACCO, M.M. (1983). Effect of PHA stimulated human lymphocytes on isolated rat atria. Participation of lipoxygenase products of arachidonic acid metabolism. *Naunyn-Schmiedebergs Arch. Pharmac*, **324**, 58–63.

STERIN-BORDA, L., CANGA, L., BORDA, E., GIMENO, M.F. & GIMENO, A.L. (1980). Inotropic effect of PGI_2 on isolated atria at different contraction frequencies. *Naunyn-Schmiedebergs Arch. Pharmac.*, **313**, 95–100.

STERIN-BORDA, L., CANGA, L. & GIMENO, A.L. (1981). Stimulating effect of TXB_2 on isolated rat atria. *Experientia*, , 592–593.

STERIN-BORDA, L., CANGA, L., GIMENO, M.F. & GIMENO, A.L. (1979). An adrenergic participation subserving a positive inotropism and chronotropism of PGI_2 on isolated rat atria. *Experientia*, **35**, 529–530.

VADLAMUDI, R.V., RODGERS, R.L. & McNEILL, J.H. (1982). The effect of chronic alloxan and streptozotocin-induced diabetes on isolated rat heart performance. *Can. J. Physiol. Pharmac.*, **60**, 902–911.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. II Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Archs. int. Pharmacodyn. Thér.*, **143**, 299–319.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature (New Biol.)*, **231**, 232–235.

WAGNER, J. & BRODDE, D.E. (1978). On the presence and distribution of adrenoceptors in the heart of various mammalian species. *Naunyn-Schmiedebergs Arch. Pharmac.*, **302**, 239–254.

(Received March 19, 1985
Revised August 12, 1985.
Accepted September 6, 1985.)