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α_1-Adrenoceptor stimulation inhibits the isoproterenol-induced effects on myocardial contractility and protein phosphorylation

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Abstract

In the present study the influence of α_1-adrenoceptor stimulation on the β-adrenoceptor agonist-induced increases in contractile parameters and protein phosphorylation was determined in isolated perfused hearts and isolated cardiac myocytes, respectively. Methoxamine inhibited the isoproterenol-induced increases in left ventricular pressure and heart rate dose dependently up to 90% and 75%, respectively; the EC_{50} of this antiadrenergic effect was 4.4 μM. The α_1-adrenoceptor antagonist, prazosin (1 μM), greatly diminished methoxamine's inhibitory action, confirming the α_1-adrenoceptor-mediated mechanism. The inotropic effect of glucagon was inhibited by methoxamine in a similar manner. Radioligand binding assays with [^{3}H]dihydroalprenolol demonstrated that the antiadrenergic action of methoxamine is not due to an unspecific β-adrenoceptor blocking property. In an additional experimental series the effects of methoxamine and isoproterenol on the protein phosphorylation pattern of isolated cardiac myocytes were investigated. Isoproterenol increased the phosphorylation state of five proteins (6-kDa, phospholamban; 15-kDa; 28-kDa, troponin I; 97-kDa; 140-kDa) while in the experiments with methoxamine the 15-kDa protein was the only phosphorylated substrate. In the presence of methoxamine the isoproterenol-induced phosphorylation of phospholamban, troponin I and the 97-kDa and 140-kDa protein was markedly inhibited while the phosphorylation state of the 15-kDa protein remained unaltered. The present study clearly demonstrated that α_1-adrenoceptor stimulation potently inhibits the β-adrenoceptor-mediated changes in contractile force and phosphorylation of key regulatory proteins, most likely through modulation of cAMP metabolism.

Keywords: Heart, rat; α_1-Adrenoceptor; β-Adrenoceptor; Myocardial contractility; Protein phosphorylation

1. Introduction

Stimulation of the heart by either α_1- or β-adrenoceptors is associated with increases of ventricular contractile force although there are qualitative differences in the mechanical response to these agents (Endoh, 1991; Terzic et al., 1993). While β-adrenoceptor stimulation induces the major contractile effect, α_1-adrenoceptors mediate a moderate and delayed inotropic response. Major differences exist between the influence of α_1- and β-adrenoceptors on the heart rate: in contrast to β-adrenoceptors, α_1-adrenoceptors mediate no chronotropic effects in adult hearts (Wagner and Brodde, 1978; Osnes et al., 1985).

The mechanism of action of β-adrenoceptor agonists is well established and involves stimulation of the adenylyl cyclase, cAMP-mediated activation of protein kinases and subsequent opening of L-type Ca^{2+} channels (Katz, 1983; Sperelakis and Wahler, 1988; Trautwein et al., 1990). α-Adrenoceptor agonists, on the other hand, are known to act via α_1-adrenoceptors present on the sarcolemma of several species including the rat (Brückner et al., 1985). Details of the signal transduction pathway, however, finally resulting in the positive inotropic response are not fully clear. α_1-Adrenoceptor stimulation occurs without increases in myocardial cAMP levels but is associated with activation of phospholipase C and formation of diacylglycerol and inositol 1,4,5-trisphosphate (IP_3) as well as activation of protein kinase C and phosphorylation of a

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sarcolemmal 15-kDa protein (Henrich and Simpson, 1988; Hartmann and Schrader, 1992; Lindemann, 1986; Brodde et al., 1978; Brückner et al., 1978).

Furthermore α_1-adrenoceptor stimulation has been shown to be involved in the regulation of cAMP levels in cardiac tissue. Watanabe and coworkers were the first to demonstrate in rat cardiac myocytes that stimulation of α_1-adrenoceptors inhibited the increase in cAMP mediated by β-adrenoceptors (Watanabe et al., 1977). Possible mechanisms suggested include α_1-adrenoceptor-mediated stimulation of cAMP phosphodiesterase activity (Buxton and Brunton, 1985,1986) and inhibition of β-adrenoceptor-stimulated cAMP accumulation by coupling to a guanine nucleotide inhibitory protein (Barrett et al., 1993).

While the inhibitory action of α_1-adrenoceptors on cAMP accumulation is a reproducible phenomenon when studied in the isolated cardiac myocyte, only little information is available about the functional consequences of simultaneous α_1- and β-adrenoceptor activation in the intact heart. Early studies demonstrated that methoxamine can inhibit the positive inotropic and chronotropic effects of isoproterenol (Imai et al., 1961a,b; Blinks, 1964,1967; James et al., 1968; Youngson and Talenik, 1985). There was, however, some disagreement about the mechanism responsible for this antagonism. Both a stimulatory effect on the cardiac α_1-adrenoceptor induced by methoxamine as well as a β-adrenoceptor blocking property have been discussed.

The present study was designed to investigate whether, and to what extent α_1-adrenoceptor stimulation of isolated rat hearts can modulate the contractile responsiveness of the heart to β-adrenoceptor stimulation. In order to circumvent α-adrenoceptor-mediated coronary vasoconstriction, the hearts were perfused at constant flow while coronary vessels were maximally dilated with sodium nitroprusside. To further understand the interaction between α_1- and β-adrenoceptor stimulation, the cAMP-dependent and independent phosphorylation of key regulatory proteins was investigated in isolated rat cardiac myocytes. Furthermore various control experiments were performed to determine the α_1-adrenoceptor specifity of methoxamine in the rat heart.

2. Materials and methods

2.1. Isolated perfused hearts

Hearts from rats (300–400 g) were perfused according to the Langendorff technique with a constant flow of 10 ml/min. A modified Krebs-Henseleit solution gassed with 95% O_2-5% CO_2 consisting of (mM) NaCl 116, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.17, $NaHCO_3$ 25, $CaCl_2$ 2.5, glucose 8.3 and pyruvate 2.0 (pH 7.4) was used. Left ventricular pressure, dP/dt_{max} and heart rate were determined via a small rubber balloon connected to a pressure transducer; coronary perfusion pressure was measured with a pressure transducer connected to the perfusion cannula. Hemodynamic parameters were recorded on a chart recorder. Substances to be tested were infused (< 0.1 ml/min) or applied as a bolus (50 μl) directly into the perfusion cannula. Infusion of methoxamine was started for at least 3 min before further interventions to reach steady state conditions. To abolish the potent vasoconstrictor effect of methoxamine, the coronary system was maximally vasodilated by use of sodium nitroprusside (10 μg/ml). In some of the experimental series hearts were electrically paced at a rate of 325 beats/min as indicated in the legends of the corresponding figures.

2.2. Cardiac membranes

Isolation of cardiac membranes was performed essentially as described (Khatter et al., 1984). In short, ventricular tissue was homogenized in four volumes of a buffer consisting of (mM) sucrose 0.25, Tris 5 and $MgCl_2$ 1 (pH 7.4). Homogenates were diluted with an equal volume of KCl (1 M), stirred (10 min) and filtered through gauze. Following the first centrifugation step ($700 \times g$; 15 min), the supernatant was spun at $10000 \times g$ (15 min) and then at $40000 \times g$ (30 min). The membrane pellet was dissolved in a buffer consisting of (mM) Tris 50, $MgCl_2$ 10 (pH 7.4; 1.5 mg protein/ml). All steps were performed at 4°C.

2.3. Radioligand binding assays

β-Adrenoceptor binding assays were performed in 250 μl containing 100 μg membrane protein and [^{3}H]dihydroalpenolol (1 nM) in a buffer consisting of (mM): Tris 50, $MgCl_2$ 10 (pH 8.0). Specific binding was defined as the portion displaceable by (−)-propranolol (1 μM). Incubation was performed at 30°C for 20 min; bound ligand was separated by rapid vacuum filtration through Whatman GF/B filters. The filters were then washed with 15 ml of incubation buffer (4°C) and radioactivity was determined by liquid scintillation counting.

2.4. Cardiac myocytes

Cells were isolated as recently described (Hartmann and Schrader, 1992). In short, the hearts were perfused with buffer I consisting of (mM): NaCl 112.6, KCl 15, KH_2PO_4 1.2, $MgSO_4$ 1.2, Hepes 10, glucose 5.5 (pH 7.4; gassed with 100% O_2). Following a non-recirculating perfusion for 10 min the hearts were perfused with

the same buffer supplemented with collagenase (0.1%) and albumin (0.5%) for an additional 50 min in a recirculating manner. After chopping, the tissue was incubated in a rotating water-bath for 30 min and $CaCl_2$ was continuously added to a final concentration of 1 mM within the last 15 min. Following sieving through nylon mesh (200 μm) the suspension was layered on 10 ml of buffer II consisting of (mM): NaCl 125, KCl 2.6, $MgSO_4$ 1.2, Hepes 10, glucose 5.5, $CaCl_2$ 1.0, supplemented with 4% albumin and was centrifuged (75 s, 400 rpm, Haereus minifuge). This centrifugation step was repeated twice with the resuspended pellet. The centrifugation step was repeated with albumin-free buffer II and the pellet (10–15 mg protein) was resuspended in 1 ml buffer II supplemented with adenosine deaminase (1 U/ml). 60–80% of cardiac myocytes were intact as determined by the rod shape criterion.

2.5. Protein phosphorylation

$^{32}P_i$, 200 μCi, was added to the suspension of cardiac myocytes. After a 60-min incubation the suspension was diluted with 10 ml of buffer II. Aliquots, 300 μl, were incubated with the substances to be tested for the indicated times. The reaction was stopped by the addition of 150 μl buffer consisting of (mM): Tris 30, EDTA 15, sodium dodecyl sulfate 9%, glycerol 15%. Before boiling for 5 min mercaptoethanol (50 μl; 25%) and bromophenolblue (10 μl; 0.1%) were added.

2.6. Electrophoresis

Samples were put on 5–15% and 10–20% linear gradient gels (Laemmli, 1970). After staining (coomassie R 250 0.125%, methanol 50%, acetic acid 10%), destaining (1, with methanol 50%, acetic acid 10% and 2, with methanol 5%, acetic acid 7%) and equilibration (methanol 40%, glycerol 5%) the gels were dried at 40°C under vacuum. Phosphoproteins were detected using Kodak X-Omat AR film and were scanned by means of a laser densitometer (LKB; Ultroscan I). Linearity between radioactivity and absorbance was shown in control experiments.

2.7. Materials

The sources of chemicals were as follows: methoxamine hydrochloride, phenylephrine hydrochloride, isoproterenol hydrochloride and prazosin hydrochloride, (−)-propranolol hydrochloride (Sigma). $^{32}P_i$; *l*-[propyl-2,3-^{3}H]dihydroalprenolol (Amersham); sodium nitroprusside (Schwarz, Germany). All other chemicals were of the highest available quality.

3. Results

In a first series of experiments the influence of methoxamine on the isoproterenol-induced increases in left ventricular pressure was determined. Basal contractile parameters, which were unaltered during maximal sodium nitroprusside vasodilation, were: left ventricular pressure 64 ± 7 mm Hg, dP/dt_{max} 1417 ± 291 mm Hg (mean ± S.E.; $n = 5$). Coronary perfusion pressure decreased in the presence of sodium nitroprusside from 78 ± 12 cm H_2O to 67 ± 9 cm H_2O (mean ± S.E.; $n = 5$). As shown by results of a representative experiment in Fig. 1, methoxamine dose dependently inhibited the isoproterenol-mediated increase in left ventricular pressure and dP/dt_{max}. Since the coronary system was maximally vasodilated, no increase in coronary perfusion pressure occurred on α_1-adrenoceptor stimulation. The isoproterenol-induced increase in contractility, however, was accompanied by an increase in coronary perfusion pressure. Statistical evaluation of the experiments revealed that methoxamine (1–100 μM) alone increased left ventricular pressure by only 15 mm Hg (Fig. 2A). Isoproterenol (20 pmol) increased left ventricular pressure by 69 ± 11 mm Hg (mean ± S.E.; $n = 3$) above basal conditions. Fig. 2B shows the dose-response curve of the methoxamine-induced inhibition of this isoproterenol-increased left ventricular

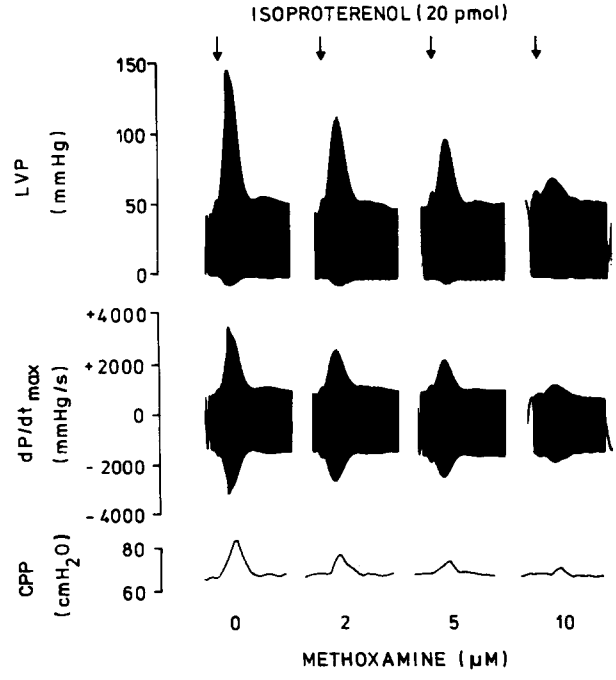

Fig. 1. Representative recording of effect of methoxamine on the isoproterenol (bolus; 20 pmol)-induced increase in left ventricular pressure (LVP), dP/dt_{max} and coronary perfusion pressure (CPP). Hearts were maximally vasodilated with sodium nitroprusside (10 μg/ml) and electrically paced at a rate of 325/min. Isoproterenol was applied as a bolus (20 pmol).

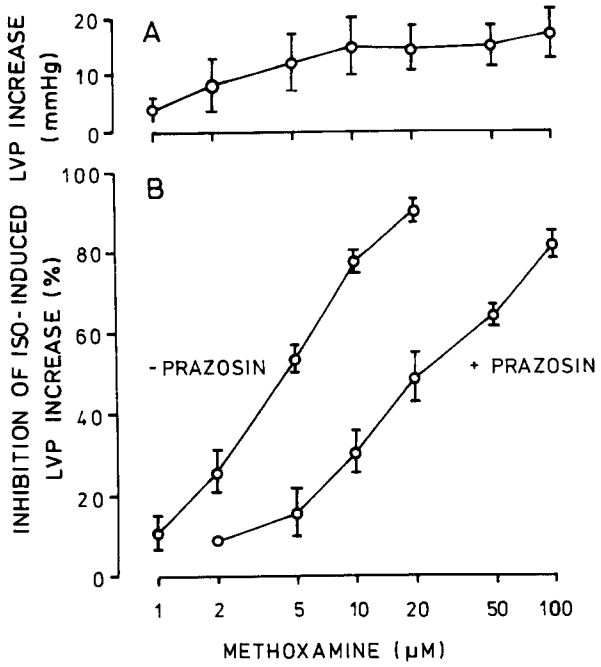

Fig. 2. (A) Effect of methoxamine on the left ventricular pressure development (LVP). Means ± S.E.; $n = 3$. (B) Effect of methoxamine on the isoproterenol (ISO; bolus 20 pmol)-induced increase in left ventricular pressure (LVP) in the absence and presence of prazosin (1 μM). Means ± S.E.; $n = 3$. For details of experimental conditions see Fig. 1.

pressure. In presence of the α_1-adrenoceptor agonist inhibition of maximally 90% could be observed and the EC_{50} was 4.4 μM. In presence of the α_1-adrenoceptor antagonist, prazosin, which had no effects on either basal or isoproterenol-stimulated contractility (data not shown), the effect of methoxamine to antagonize the inotropic action of isoproterenol was significantly inhibited; the dose-response curve shifted to 5-fold higher concentrations (EC_{50}: 22 μM; Fig. 2B). Additional experiments revealed that the methoxamine-induced inhibition of the isoproterenol-mediated increase in cardiac contractility was comparable whether sodium nitroprusside was present or absent (data not shown). Furthermore the effect of methoxamine on the positive inotropic effect of glucagon, which is also known to be mediated by cAMP, was investigated. The increases in left ventricular pressure induced by equipotent doses of glucagon and isoproterenol, respectively, were inhibited to a quite similar degree in the presence of methoxamine (Fig. 3).

In further experiments the effect of methoxamine on the heart rate under basal and isoproterenol-stimulated conditions was investigated. Methoxamine (10 μM) inhibited the positive chronotropic effect of isoproterenol (20 pmol) to about 75%, while the heart rate under control conditions was not altered by the α_1-adrenoceptor agonist (Fig. 4).

Since the specificity of methoxamine is crucial for

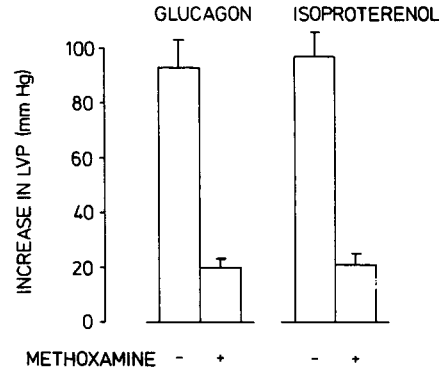

Fig. 3. Effect of methoxamine (10 μM) on the increase in left ventricular pressure induced by glucagon (175 nmol) and isoproterenol (20 pmol), respectively. Means ± S.E.; $n = 3$.

the interpretation of the data of the present study, the affinity of methoxamine to cardiac β-adrenoceptors was determined in radioligand binding assays, using the β-adrenoceptor antagonist, [^{3}H]dihydroalprenolol. Binding of the radioligand to ventricular membranes was saturable and was displaced by (−)-propranolol; Scatchard plots showed a receptor density of 116 fmol/mg protein and a K_D of 1.2 nM. The threshold for the inhibition of [^{3}H]dihydroalprenolol binding induced by (−)-propranolol and methoxamine, was 1 nM and 100 μM, respectively (Fig. 5).

In a second experimental series, $^{32}P_i$-prelabelled isolated cardiac myocytes from rat heart were incubated with isoproterenol (1 μM) in the absence and presence of methoxamine (1–100 μM). The protein phosphorylation pattern was determined by autoradiography of cellular proteins resolved by sodium dodecyl sulfate gel electrophoresis. Resolution of phosphorylated proteins on a 5–15% gel showed that isoproterenol induced the phosphorylation of four pro-

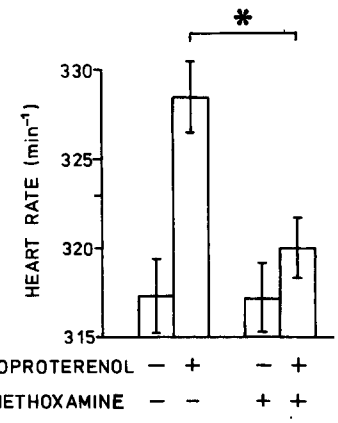

Fig. 4. Effect of methoxamine (10 μM) on the isoproterenol (20 pmol)-induced increase in heart rate. The coronary system was maximally vasodilated in the experiments with sodium nitroprusside (10 μg/ml). Isoproterenol was given as a bolus (20 pmol). Means ± S.E.; $n = 3$; $^*P < 0.01$.

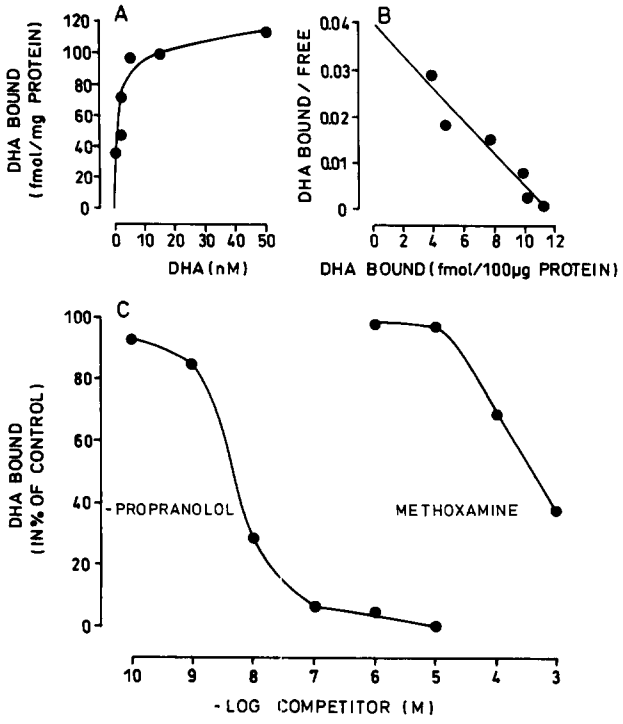

Fig. 5. β-Adrenoceptor-binding studies with ^{3}H-dihydroalprenolol. (A) *l*-[^{3}H]dihydroalprenolol (DHA) binding as a function of the concentration of ligand. (B) Scatchard plot. (C) Inhibition of *l*-[^{3}H]dihydroalprenolol (1 nM) binding by (−)-propranolol and methoxamine.

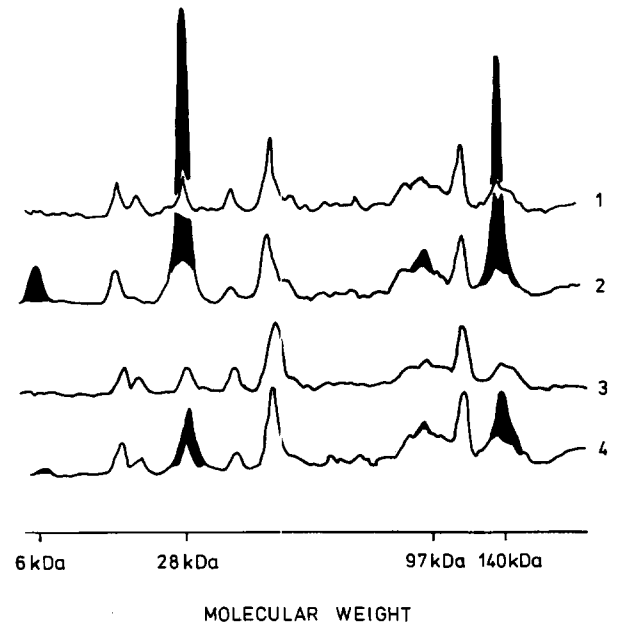

Fig. 6. Densitometric scans of autoradiograms obtained from intact cell phosphorylation experiments performed in the presence and absence of isoproterenol and methoxamine (for details see Methods). Cellular proteins were separated on a 5–15% linear gradient gel. Black areas show increases in optical density caused by isoproterenol. Lane 1: control conditions. Lane 2: isoproterenol stimulation (2 min; 1 µM). Lane 3: methoxamine stimulation (5 min; 10 µM). Lane 4: combined stimulation with methoxamine (5 min; 10 µM) and isoproterenol (2 min; 1 µM).

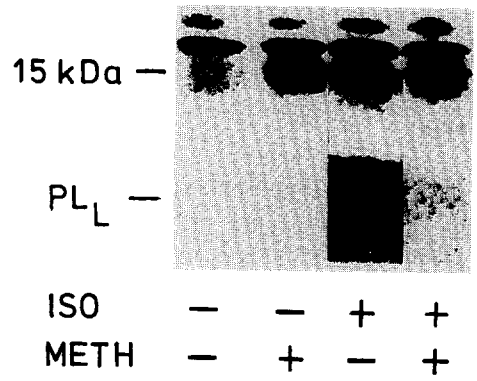

Fig. 7. Autoradiogram demonstrating the influence of methoxamine (5 min; 10 µM) and isoproterenol (2 min; 1 µM) on protein phosphorylation of intact cardiac myocytes resolved by a 10–20% linear gradient gel. The figure shows the region of interest corresponding to the 15-kDa protein and phospholamban.

teins (6-, 28-, 97-, 140-kDa); methoxamine did not detectably alter the phosphorylation pattern compared to control levels in this electrophoretic system (Fig. 6, lane 1–3). In the presence of methoxamine the isoproterenol-induced phosphorylation of the four proteins was greatly diminished (Fig. 6, lane 4). Furthermore, analysis of the same protein samples using a 10–20% gel led to the separation of 15-kDa protein which was phosphorylated by both α_1- and β-adrenoceptor stimulation, respectively (Fig. 7). Additionally, Fig. 7 shows that the β-adrenoceptor-induced 15-kDa protein phosphorylation was apparently not altered in presence of methoxamine, while the phosphorylation of the 6-kDa protein was markedly inhibited.

The statistical evaluation of the data shown in Fig. 8 demonstrated that methoxamine (1–100 µM) inhibited the isoproterenol (1 µM)-induced phosphorylation of substrates corresponding to 6-, 28-, 97- and 140-kDa. Inhibition was about 80% at high methoxamine con-

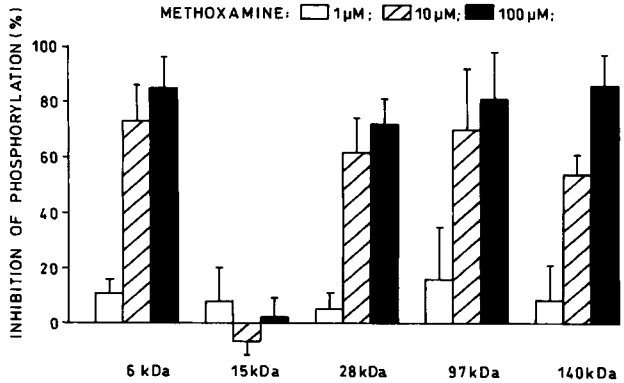

Fig. 8. Effect of methoxamine (1–100 µM) on the isoproterenol (1 µM) induced increases in phosphorylation state of substrates corresponding to 6-, 15-, 28-, 97- and 140-kDa. Means ± S.E.; $n = 3$.

centrations. The phosphorylation state of the 15-kDa protein, however, remained unaltered.

4. Discussion

The results of the present study clearly demonstrated that α_1-adrenoceptor stimulation of the isolated perfused rat heart exerts a potent antiadrenergic action. Methoxamine attenuated the isoproterenol-induced increase in ventricular force development and chronotropy by 90% and 75%, respectively. The inhibitory action of methoxamine could also be observed on two regulatory proteins: the isoproterenol-induced phosphorylation of phospholamban and troponin I was strongly inhibited by methoxamine.

Various control experiments were performed to demonstrate that the potent antiadrenergic action of methoxamine is a specific effect. Firstly, the α_1-adrenoceptor antagonist, prazosin, inhibited the isoproterenol antagonistic effect of methoxamine with a K_B of 220 nM. It is, however, important to note, that due to α_1-adrenoceptor subtypes, the affinity of prazosin reported in the literature widely varies showing dissociation constants in the range of 0.2–800 nM (Muramatsu et al., 1990; Yazawa et al., 1992). Secondly, volume-constant perfusion of a maximally vasodilated coronary vascular bed insured that α_1-adrenoceptor-mediated vasoconstriction resulting in hypoperfusion was eliminated. It should be emphasized, however, that similar results were obtained in the absence of sodium nitroprusside, thereby excluding a direct effect of the vasodilator on the isoproterenol-antagonistic action of methoxamine. Furthermore, a significant β-adrenoceptor-blocking effect of methoxamine could be excluded by radioligand binding assays with [^{3}H]dihydroalprenolol, demonstrating that displacement of the ligand occurred only at methoxamine concentrations ≥ 100 μM.

Further insights into the mechanism of the antiadrenergic action of methoxamine comes from the experiments with glucagon, which – like isoproterenol – acts via cAMP (Murad and Vaughan, 1969; Méry et al., 1990). The positive inotropic action of glucagon and isoproterenol was equally well inhibited by methoxamine. Since it is very unlikely that the glucagon receptor is blocked by methoxamine, the results provide further evidence for an α_1-adrenoceptor-mediated mechanism and the involvement of cAMP. Moreover this notion is supported by results of a recent study of Barrett and coworkers, who demonstrated that in rat neonatal cardiac myocytes the increase in cAMP levels induced by β-adrenoceptor stimulation is inhibited by activation of α_1-adrenoceptors which are coupled to G_i-proteins (Barrett et al., 1993).

Consistent with data in the literature, the positive inotropic effect of methoxamine in the rat heart was slight and amounted to only 15 mm Hg above basal (Otani et al., 1988; Osnes et al., 1978). This effect does not involve cAMP (Brodde et al., 1978; Brückner et al., 1978) but was shown by others to be associated to increases in the activity of phospholipase C, followed by the formation of IP_3 and 1,2-diacylglycerol (Henrich and Simpson, 1988; Scholz et al., 1988). While IP_3 may mobilise Ca^{2+} from the sarcoplasmic reticulum, 1,2-diacylglycerol has been shown to activate protein kinase C (Brückner et al., 1985; Berridge and Irvine, 1984). We have recently shown that, in intact cardiac myocytes, protein kinase C phosphorylates a membrane-bound 15-kDa protein which has been postulated to be involved in the inotropic action of α_1-adrenoceptor stimulation (Hartmann and Schrader, 1992; Lindemann, 1986). Similarly, the present study demonstrated that this 15-kDa substrate is the only protein phosphorylated by methoxamine.

Based on the sarcolemmal origin as well as on the correlation between phosphorylation state and inotropic response to catecholamines a functional relationship of the 15-kDa protein to contractile force was postulated (Lindemann, 1986). The protein sequence of the 15-kDa protein was recently determined and it has been shown that it can be phosphorylated by protein kinase C and cAMP-dependent protein kinases (Palmer et al., 1991). Consistent with this is our observation that both methoxamine and isoproterenol phosphorylated the 15-kDa protein in rat cardiac myocytes. However, the isoproterenol-induced phosphorylation of this protein was not attenuated by methoxamine as was contractility. It is also remarkable that phorbol 12-myristate 13-acetate, which phosphorylates the 15-kDa protein, exerts a negative inotropic action in the intact heart (Leatherman et al., 1987; Karmazyn et al., 1990). Collectively, the dissociation between phosphorylation state and contractile force development observed by us and others suggests that the 15-kDa protein may not be a crucial step in mediating the functional effects of adrenoceptor stimulation. However, further investigation is necessary to define the function of the 15-kDa protein.

Although details of the interaction between the α_1- and β-adrenoceptor-mediated signal transduction pathway have not been worked out, our experimental results as well as findings in the literature (Watanabe et al., 1977; Buxton and Brunton, 1985,1986; Barrett et al., 1993) argue for a prominent role of the adenylyl cyclase system in this inhibitory process. β-Adrenoceptor stimulation caused the phosphorylation of five proteins with 6-, 15-, 28-, 97- and 140-kDa most likely mediated through activation by cAMP-dependent protein kinases (Murray et al., 1989). With the exception of the 15-kDa protein (see above) the phosphorylation of the remaining four phosphoproteins was inhibited

by methoxamine up to 90%. Interestingly, the concentration of the α_1-adrenoceptor agonist required (1-100 μM methoxamine) to cause inhibition of phosphorylation was identical with the hemodynamic potency of methoxamine (compare data of Figs. 2 and 8). Our results cannot, however, exclude the possibility that α_1-adrenoceptors could, in addition, modulate β-adrenoceptor signalling through well recognised pathways involving the inositol phosphates and diacylglycerol.

The cAMP-dependent phosphorylation of three cardiac proteins has been suggested to be involved in the inotropic response to β-adrenoceptor stimulation. Among the well characterised substrates, phospholamban, the 6-kDa protein, can stimulate Ca^{2+} uptake by the sarcoplasmic reticulum when phosphorylated and can explain the enhanced rate of relaxation associated with β-adrenoceptor stimulation of the heart (Movsesian et al., 1984; Simmerman et al., 1986). In the case of troponin I, the 28-kDa protein, phosphorylation has been shown to alter myofilament sensitivity to Ca^{2+}, which would also promote diastolic relaxation (Katz, 1979; Kranias and Solaro, 1982). While the C-protein (140-kDa) has been shown to be a good in vitro substrate for cAMP-dependent protein kinase, the functional relevance of this protein is currently unclear (Garvey et al., 1988). Finally, cAMP-dependent phosphorylation of L-type Ca^{2+} channels most likely is responsible for the enhanced Ca^{2+} entry on β-adrenoceptor stimulation (Trautwein et al., 1990). Collectively, these data suggest that cAMP is a positive inotropic agent, the effects of which are mediated by protein phosphorylation, however the current evidence is not conclusive (Murray et al., 1989). Nevertheless, the parallelism found in the present study between phosphorylation state and contractile response on α_1-adrenoceptor stimulation argues for causal role.

The extent of α_1-adrenoceptor-mediated inhibition of β-adrenoceptor-induced increases in cardiac contractility may be of functional significance. In two recent studies stimulation of myocardial α_1-adrenoceptors was shown to exert a positive inotropic effect in the human heart in vivo also (Curiel et al., 1989; Landzberg et al., 1991). It was proposed that the positive inotropic effect of α_1-stimulation may complement that caused by β-adrenoceptor stimulation. Similarly, myocardial α_1-adrenoceptors were considered as a reserve mechanism to maintain myocardial responsiveness under conditions where β-adrenoceptor function is compromised (for review see Terzic et al., 1993). The concept of an additive contractile action of α_1- and β-adrenoceptor stimulation, however, may not hold true. It may be speculated that, due to the potent antagonism between α_1- and β-adrenoceptor stimulation demonstrated in this study, sole stimulation of α_1-adrenoceptors may antagonise the sympathetic drive to the heart through inhibition of β-adrenoceptor stimulation. Alternatively, the simultaneous activation of cardiac α_1- and β-adrenoceptors by noradrenaline may provide a feedback mechanism preventing excessive β-adrenoceptor stimulation of the heart.

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