receptors such that an inhibitory influence on hypothalamic DA neurones is reduced and prolactin secretion inhibited. Our results are consistent with GABAergic modulation of dopaminergic transmission within the hypothalamus and with the observed changes in prolactin secretion, but they do not preclude effects of muscimol at GABAergic receptors which directly modulate prolactin secretion[20,21].

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Evidence against a role of $(Na^+ + K^+)$-ATPase in the alpha-adrenoceptor mediated positive inotropic effect of phenylephrine[1]

W. Schmitz, H. Scholz[2], E. Erdmann and K. Werdan

Abteilung III, Biochemische Pharmakologie, Institut für Pharmakologie und Toxikologie, Medizinische Hochschule Hannover, D-3000 Hannover 61 (Federal Republic of Germany) and Medizinische Klinik I, Klinikum Grosshadern, Universität München, D-8000 München 70 (Federal Republic of Germany), 18 October 1979

Summary. Phenylephrine (0.1–100 μM) in the presence of 1 μM propranolol increased the force of contraction in electrically driven papillary muscles from cats. This presumably alpha-adrenoceptor mediated positive inotropic effect of phenylephrine occurred without any influence on $(Na^+ + K^+)$-ATPase activity.

It is generally accepted that the positive inotropic response to adrenergic agents in the heart is mediated predominantly by beta-adrenoceptors. However, evidence is increasing that alpha-adrenoceptors are also present in the myocardium and that positive inotropic effects may be produced by stimulation of these sites[3–5]. These 2 inotropic effects are qualitatively different from each other. The alpha-adrenergic positive inotropic response, for instance, appears to be independent of the cAMP-system[3–5]. In contrast, the increase in myocardial force of contraction due to alpha-adrenoceptor stimulation, but not that due to beta-adrenoceptor stimulation, is critically dependent on the frequency of stimulation[6–8]. Such a frequency-dependence has also been reported for cardiac glycosides[9] which, in turn, are widely believed to produce their positive inotropic effects via an inhibition of the $(Na^+ + K^+)$-ATPase activity[10]. With this in mind, the present experiments were designed to investigate whether an inhibition of $(Na^+ + K^+)$-ATPase activity might be involved in the alpha-adrenergic positive inotropic response. The effect of phenylephrine on myocardial force of contraction and on $(Na^+ + K^+)$-ATPase activity was studied under identical conditions. All experiments were performed in the presence of propranolol in order to minimize interference from beta-adrenoceptors.

Materials and methods. Cats (b.wt 0.8–2.0 kg) were anaesthetized with sodium pentobarbital (30 mg per kg i.p.) and papillary muscles (diameter 1 mm or less) were dissected from the right ventricles. The preparations were attached to a platinum stimulating electrode and mounted individually in glass tissue chambers for recording isometric contractions as described previously[11]. The bathing solution (50 ml) containing (mM) NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.5 was equilibrated with 95% O_2 + 5% CO_2 and maintained at 35 °C, pH 7.4. The preparations were driven electrically at a frequency of 0.2 Hz (duration 5 msec, intensity about 10% above threshold). Drugs used were (−)-phenylephrine HCl (Boehringer Ingelheim) and (±)-propranolol HCl (ICI). The compounds were freshly dissolved in bathing medium. Phenylephrine concentration-response curves were obtained cumulatively; the time of exposure to each concentration was 15 min. Propranolol (1 μM) was added 30 min before phenylephrine and was present during the entire experiment. We have shown previously[5] that beta-adrenoceptors are sufficiently blocked under these conditions.

After the papillary muscles had been dissected from the hearts, the remaining ventricular tissue was frozen and kept at −60 °C until used. After thawing, $(Na^+ + K^+)$-ATPase (EC 3.6.1.3) was prepared as described by Pitts and Schwartz[12] but without glycerol treatment. Enzyme activity was determined by the coupled optical assay in an ATP-regenerating system[13]. The incubation medium (buffered with $NaHCO_3$ to pH 7.4) was the same as that used for the

determination of force of contraction; the total volume was 2 ml. The enzyme protein was incubated in the medium for 15 min at 37 °C in each test tube with 1 μM propranolol and different concentrations of phenylephrine. Then 0.4 ml of this mixture were assayed rapidly for ATPase activity in the coupled optical assay with continuous recording for 10 min at least. The oxidation of NADH was linear during this time. Under these conditions, 97% of total ATPase activity could be inhibited by 100 μM ouabain and thus was defined as $(Na^+ + K^+)$-ATPase. 1 enzyme unit is defined as 1 μmole ATP hydrolyzed per min at 37 °C. Protein was determined by the method of Lowry et al.[14]. All assays were carried out in duplicate.

In order to estimate $(Na^+ + K^+)$-ATPase activity in intact preparations as well, we studied the effect of phenylephrine in the presence of propranolol on ouabain-sensitive $^{86}Rb^+$-uptake. The experiments were performed as described by Yamamoto et al.[15] with some modifications. Papillary muscles were treated in exactly the same way as described above for the measurement of force of contraction. 10 min after the application of phenylephrine, trace amounts of $^{86}RbCl$ (5 μCi; about 10^7 cpm) were added to the incubation medium. After incubation for 10 min in the presence of $^{86}Rb^+$, the papillary muscles were rinsed for 10 sec in tracer-free bathing medium at 4 °C, blotted on filter paper (for 3 min) and weighed. The amount of radioactivity in the tissue was assayed in a scintillation counter (Packard Tricarb 2660) after dissolving each preparation in 1 ml Soluene-350 (Packard) at 60 °C for 60 min and addition of 10 ml scintillation fluid (Unisolve, Zinsser). $^{86}Rb^+$-uptake was linear for at least 60 min and was maximally inhibited by 5 μM ouabain under these conditions. $^{86}Rb^+$ was used as a tracer to calculate the amount of $(^{86}Rb^+ + K^+)$-uptake quantitatively. The basal $(^{86}Rb^+ + K^+)$-uptake was 249 ± 37 nmoles/100 mg wet wt per min ($\bar{x} \pm$ SEM).

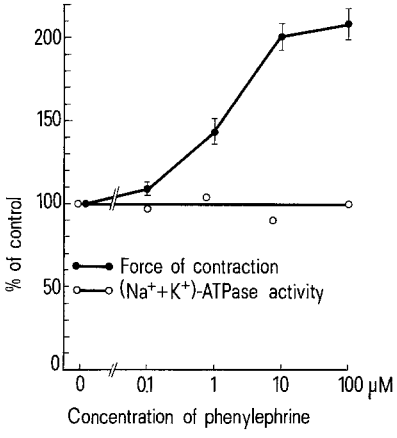

Fig. 1. Effect of phenylephrine (0.1–100 μM) in the presence of 1 μM propranolol on force of contraction (●) of isolated electrically driven papillary muscles and on $(Na^+ + K^+)$-ATPase activity (○) of heart ventricular cell membranes (0.24 mg protein) from cats. Force of contraction and $(Na^+ + K^+)$-ATPase activity are given in percent of basal values measured in the absence of phenylephrine. ●, means ± SEM of 5 experiments. ○, means of duplicate determinations; similar results were obtained in 2 other separate experiments. Control values were 13.3 ± 2.4 mN · mm^{-2} (●, N = 5) and 0.23 units · mg^{-1} membrane protein (○), respectively.

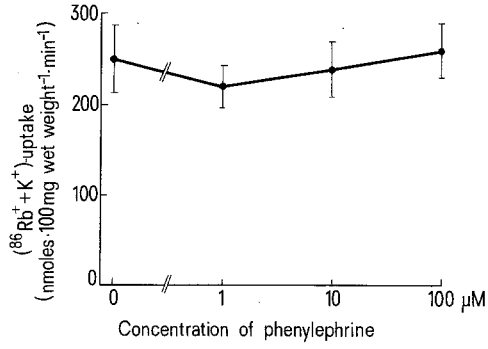

Fig. 2. Effect of phenylephrine (1–100 μM) in the presence of 1 μM propranolol on $(^{86}Rb^+ + K^+)$-uptake of electrically driven papillary muscles isolated from cats. Values are means ± SEM of 3 experiments each.

Results and discussion. Figure 1 shows that phenylephrine in the presence of 1 μM propranolol increased the force of contraction of the papillary muscles in a concentration-dependent manner. The positive inotropic effect of phenylephrine became significant at 0.1 μM and was maximal at 100 μM with a more than 2-fold increase in force of contraction over pre-drug control.

It is also evident from figure 1 that this effect was not accompanied by an inhibition of $(Na^+ + K^+)$-ATPase activity. Phenylephrine did not produce any detectable change in $(Na^+ + K^+)$-ATPase activity at any concentration studied.

One might argue that the failure of phenylephrine to inhibit isolated $(Na^+ + K^+)$-ATPase is inconclusive because the integrity of the alpha-adrenoceptors might have been destroyed by the $(Na^+ + K^+)$-ATPase isolation procedure. However, this can probably be excluded because phenylephrine in the presence of propanolol also failed to inhibit $(^{86}Rb^+ + K^+)$-uptake, a measure of $(Na^+ + K^+)$-ATPase activity, in intact papillary muscles (figure 2). In these experiments the force of contraction was increased about 2-fold as it was in the experiments described in figure 1 so that the integrity of the alpha-adrenoceptors can be taken to be maintained. The maintained responsiveness of the $(Na^+ + K^+)$-ATPase can be deduced from the finding that $(^{86}Rb^+ + K^+)$-uptake was maximally inhibited by 5 μM ouabain as mentioned above.

We have shown previously[5] that the positive inotropic effect of phenylephrine obtained under the present conditions is antagonized by phentolamine and is thus likely to be mediated by a stimulation of alpha-adrenoceptors. This alpha-adrenergic action of phenylephrine occurred without detectable increase in the cAMP levels of the preparations[5] and was not accompanied by a so-called relaxant effect[5,16] (abbreviation of systole, depression of potassium contracture) characteristically observed on beta-adrenoceptor stimulation (for literature see Tsien[17]). The present study provides evidence that the alpha-adrenergic positive inotropic effect of phenylephrine is also not due to a change in $(Na^+ + K^+)$-ATPase activity so that the mechanism underlying this action as well as the reason for its frequency dependence remain to be elucidated.

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2 Address for reprint requests: Dr H. Scholz, Abteilung III (Biochemische Pharmakologie), Institut für Pharmakologie und Toxikologie, Medizinische Hochschule Hannover, D-3000 Hannover 61.

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Newly synthesized elastin is associated with neoplastic epithelial cells in human mammary carcinoma[1]

K.G. McCullagh[2], K. Barnard, J.D. Davies and S.M. Partridge

Department of Pathology, University of Bristol, Bristol BS8 1TD (England), 11 February 1980

Summary. Indirect immunofluorescence with a purified antiserum to human foetal elastin has identified newly synthesized elastin on the membranes of neoplastic epithelial cells in human mammary carcinoma.

The focal accumulation of elastic tissue (elastosis) is a recognized histological feature of scirrhous mammary carcinoma in women[3,4]. Studies utilising elastase digestion[3] and electron microscopy[5-7] have confirmed the presence of elastin and elastic tissue microfibrils in this material. The ultrastructure of elastic fibres within the tumour mass differs from that of mature arterial elastica, the tumour fibres being thinner, randomly orientated and containing a lower ratio of amorphous elastin to microfibrils[6,7], all of which are characteristic features of newly synthesized elastic tissue[8]. The phenomenon of tumour elastosis has usually been regarded as a stromal reaction to the presence of infiltrating malignant epithelium[5,7]. However, it has also been suggested that elastic tissue may originate from the tumour cells themselves[6]. This view is supported by the results of the present study in which synthesized elastin in tumour tissues has been demonstrated by immunofluorescence to be intimately associated with neoplastic epithelial cells.

Insoluble elastin was purified from breast carcinoma tissue and from adult and foetal human aortas after decalcification in 0.5 M EDTA, 0.01 M Tris HCl, pH 7.4[9], autoclaving with distilled water and repeated extraction with 0.1 N NaOH at 98 °C[10]. Amino acid analyses of these preparations are shown in the table. Despite exhaustive extraction, the tumour elastin remained contaminated with small amounts of glycoprotein (about 5%). This is reflected in larger amounts of aspartate in the tumour elastin than in the pure feotal aortic elastin. The amounts of the cross linking amino acids desmosine and isodesmosine in relation to the amount of lysine give some indication of the age of the elastin. Tumour elastin is poorly cross linked (desmosines/lysine ratio = 0.35) and immature in comparison to adult aortic elastin (desmosines/lysine ratio = 0.55). Similar immaturity is observed in foetal aortic elastin (desmosines/lysine ratio = 0.25).

Using the highly purified foetal aortic elastin as an antigen, antisera were raised in sheep to both the insoluble protein and its oxalic acid solubilized peptides (α-elastin)[11]. The methods used and characteristics of the antisera produced have recently been described[12]. Purified antibodies to human foetal α-elastin did not cross-react with elastic fibre glycoproteins or purified type I and III collagens but gave strong reactions in passive haemagglutination tests with human α-elastins. The maximum reciprocal titre achieved in this system when foetal α-elastin was used as the coating antigen was 4 times that achieved with adult α-elastin, indicating preferential affinity of the antibodies for immature elastin[12].

Elastin was localized by indirect immunofluorescence in fresh frozen sections of surgically excised human tissues. Binding of the antibody in sections from child aorta resulted in bright specific fluorescence over large and small elastic lamellae (figure, a). In mammary carcinomas with

Amino acid composition of elastins extracted from human mammary carcinoma and adult and foetal human aorta (values are given as moles/1000 moles)

Amino acid	Human mammary carcinoma elastin*	Human adult aortic elastin	Human foetal aortic elastin
Hydroxyproline	16.1	18.2	12.6
Aspartic acid	11.2	11.5	3.8
Threonine	13.5	13.7	11.5
Serine	10.9	9.3	8.5
Glutamic acid	27.5	28.2	18.8
Proline	144.5	154.5	122.0
Glycine	263.3	252.1	288.8
Alanine	211.5	207.5	217.1
Valine	130.2	130.0	140.1
Methionine	3.3	5.3	2.5
Isoleucine	26.9	29.2	27.3
Leucine	65.2	68.6	65.1
Tyrosine	22.5	21.2	21.5
Phenylalanine	25.1	24.4	26.0
Lysine	12.2	9.1	10.7
Histidine	1.7	0.8	0.6
Arginine	8.6	9.7	7.1
Desmosine	2.0	2.8	1.4
Isodesmosine	1.9	2.2	1.3

* Mean of analyses from 4 elastotic tumours.