INTRODUCTION

The antidepressants are the agents that have been used extensively in the treatment of affective disorders. They are however not without side effects. Among often occurring unwanted effects are those affecting cardiovascular system, most commonly manifested as hypotension, and/or induction or worsening of hypertension (1). These may be of relevance in anaesthesia because the anaesthetic agents by themselves produce hypotension and a combination of these agents may display synergy. Indeed, if taken over prolonged time, it has been shown that during anaesthesia they may cause unpleasant complications, like serious cardiac arrhythmias (2, 3). Or, quite dramatic development is possible like the episodes of hypotension, resistant to therapy (4). The mechanisms of these agents producing hypotension are unclear. Most investigations were concerned with the synaptic transmission or with direct effects of antidepressants on smooth muscle cells (5-7). However, the direct effects of the antidepressants on peripheral circulation and in particular the role of the vascular endothelium, which is an important source of the potent vasorelaxing agent nitric oxide (NO) production, remained largely unexplored.

The present work was undertaken to investigate whether some most commonly prescribed antidepressant agents could directly influence vascular tone in an in vitro model of isolated rat aorta. Amitriptyline and fluoxetine were included in the study as agents often causing hypotension and they were contrasted with fluoxetine, an agent that normally does not affect blood pressure, and venlafaxine, an agent associated with induction or worsening of hypertension (8). Direct effects of amitriptyline and fluoxetine on arterial smooth muscle have already been reported (9-12). Venlafaxine and tranylcypromine have not been investigated for possible effects on arterial contractility in vitro.
This study was performed in accordance with the local Instructions for Animal Care of the Greifswald University and was approved by the state Commission for Animal Protection in Schwerin, Germany. Experiments were performed on aortas taken from Lewis 1A rats (in total 50 animals), weighing 250–400 g. For the experiment, rats were anesthetized by intraperitoneal application of thiopental (100 mg/kg) and by sectioning of abdominal aorta, quickly exsanguinated. The thoracic aortas were then isolated, immersed in Krebs-Henseleit solution (KH; in mM: 113 NaCl, 4.8 KCl, 1.3 MgCl₂, 1.2 KH₂PO₄, 25 NaHCO₃, 2.5 CaCl₂, 5.7 glucose) and cleaned from the surrounding tissue. In some preparations endothelium was removed mechanically by gently rubbing the interior of the aortal preparation with a moistened cotton-wrapped metal stick. Aortal ring preparations (about 2–3 mm long) were mounted in the customary manner between two stainless steel hooks, where the lower hook served as a fixed point and the upper hook was connected to the isometric force transducer (Entran, ELJ-5045C-35G, purchased from EMKA Technologies, Paris, France). The signal was amplified (STA 2808, EMKA Technologies, Paris, France) and displayed on paper recorder (Rikadenki multipen recorder, R-50 series, Hugo Sachs Elektronik, March-Hugstetten, Germany). The quickly mounted preparation was then immersed in the organ bath (20 ml) filled with KH solution. The KH solution (at 37°C, pH 7.4, gassed with 95% O₂/5% CO₂) was exchanged every 20 min. The force transducer was attached to micromanipulator which permitted displacement of the upper hook along a strict vertical axis and the adjustment of the muscle length. After a period of stabilization (60 min) the vascular muscle was stretched to its optimal length, which was established, in the preliminary experiments, to correspond to a counter-weight of 2 g (data not shown). The integrity of the endothelium in aortas was tested, at the end of the experiment, by pre-contracting the preparation with phenylephrine (0.1 µM) and, when the contraction reached a plateau, cumulative concentrations of the antidepressants were applied. The denudation by rubbing resulted in loss of function of the endothelium, as indicated by the absence of dilation to acetylcholine (data not shown).

The following agents, that in clinical use as antidepressants were examined: amitriptyline, a secondary amine tricyclic antidepressant mainly inhibiting noradrenaline uptake; tranylcypromine, an irreversible inhibitor of monoaminoxidase and inhibitor of prostanoid synthesis; fluoxetine, a selective serotonin reuptake inhibitor; venlafaxine, an inhibitor of noradrenaline and serotonin reuptake.

### Table 1. The effects of endothelium removal on half maximal effects (pEC₅₀) of phenylephrine in rat aorta after 30 minutes incubation with antidepressant agents.

<table>
<thead>
<tr>
<th>Incubation with</th>
<th>With endothelium</th>
<th>n</th>
<th>Without endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>5.73 (0.02)***</td>
<td>8</td>
<td>5.98 (0.12)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>6.93 (0.02)***</td>
<td>8</td>
<td>7.31 (0.07)</td>
</tr>
<tr>
<td>Tranylcypromine</td>
<td>7.79 (0.07)***</td>
<td>6</td>
<td>8.39 (0.07)</td>
</tr>
<tr>
<td>Control</td>
<td>6.60 (0.10)</td>
<td>12</td>
<td>7.20 (0.10)</td>
</tr>
</tbody>
</table>

After measuring isometric tension pEC₅₀ values were calculated (mean ±S.E.M; logₐEC₅₀ mol/L) of n-experiments as indicated obtained in preparations with and without endothelium. Data were taken from control and pre-incubated preparations (with either amitriptyline, fluoxetine, tranylcypromine; each 0.5 µM for 30min), which was followed by stimulation with cumulative concentrations of phenylephrine (0.5 nM - 0.5 mM). P values derived from one-way ANOVA followed by Dunnet post-hoc-test. Incubation vs. Control: ***P<0.001; preparations with vs. without endothelium: #P<0.05; ###P<0.001.

At first, a series of test for the effects of the antidepressants amitriptyline, fluoxetine, tranylcypromine or venlafaxine on induced tension were examined. Aortal rings (with and without endothelium) were incubated with the antidepressant agents, each 0.5 µM for 30 minutes (this concentration reaches approximately blood level of the agents in patients when used as therapy). Then, tension was elicited by cumulative concentrations of phenylephrine (0.5 nM–0.5 mM). Preparations without incubation with antidepressant agents served as controls.

In the second series of the experiments (with and without endothelium), the effects of the antidepressants on agonist elicited pre-contraction, were examined. The preparations were pre-contracted either with phenylephrine (0.1 µM), KCl (20 or 40 mM) or prostaglandin F₂α (5 µM). After pre-contraction, when the tension reached a plateau (maximum tension Tₘₓₚ), preparations were stimulated with cumulative concentrations of the antidepressants (0.05 µM–500 µM). The primary point of measurement was the basal tone after the stabilization period and represented 0% of Tₘₓₚ. The second point was the maximum tension Tₘₓₚ that 25 corresponds to 100%. Maximal relaxing effects of the antidepressant agents was defined as a fraction of the tension achieved in pre-contraction that was taken to be 100%. Amitriptyline, fluoxetine and tranylcypromine dilated the rat aorta beyond the basis tone resulting in negative % values.

In the third set of experiments endothelium intact preparations were pre-incubated either with NO generation inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME, 500 µM), an inhibitor of guanylyl cyclase 1H(1,2,4)oxodiazolo-(4,3-a)quinolinol-one (ODQ, 1 µM) or an inhibitor of adenyl cyclase, 9-(tetrahydro-2-furyl)-9H-purin-6-amine (SQ 22536, 100 µM), for 30 minutes. Then they were pre-contracted with phenylephrine (0.1 µM) and, when the contraction reached a plateau, cumulative concentrations of the antidepressants were applied. In the last set of experiments preparations with endothelium were incubated for 30min with the potassium channel blocking agents charybdotoxine (0.05 µM, calcium dependent K⁺ channel), glibenclamide (10 µM, ATP-dependent K channel), tetrathyrammoniumchloride (TEA, 10 mM, non-specific K⁺ channel) or 4-aminopyridine (4-AP, 100 µM, voltage operated K⁺ channel).

Some endothelium intact preparations were pre-contracted with 40 mM KCl, then incubated for 30 minutes with propranolol (10 µM, β-adrenergic blocker) or prazosine (10 µM, α₁-adrenergic blocker) before cumulative concentrations of the antidepressants would be applied.

### Experimental protocol

This study was performed in accordance with the local Instructions for Animal Care of the Greifswald University and was approved by the state Commission for Animal Protection in Schwerin, Germany. Experiments were performed on aortas taken from Lewis 1A rats (in total 50 animals), weighing 250–400 g. For the experiment, rats were anesthetized by intraperitoneal application of thiopental (100 mg/kg) and by sectioning of abdominal aorta, quickly exsanguinated.

The thoracic aortas were then isolated, immersed in Krebs-Henseleit solution (KH; in mM: 113 NaCl, 4.8 KCl, 1.3 MgCl₂, 1.2 KH₂PO₄, 25 NaHCO₃, 2.5 CaCl₂, 5.7 glucose) and cleaned from the surrounding tissue. In some preparations endothelium was removed mechanically by gently rubbing the interior of the aortal preparation with a moistened cotton-wrapped metal stick. Aortal ring preparations (about 2–3 mm long) were mounted in the customary manner between two stainless steel hooks, where the lower hook served as a fixed point and the upper hook was connected to the isometric force transducer (Entran, ELJ-5045C-35G, purchased from EMKA Technologies, Paris, France). The signal was amplified (STA 2808, EMKA Technologies, Paris, France) and displayed on paper recorder (Rikadenki multipen recorder, R-50 series, Hugo Sachs Elektronik, March-Hugstetten, Germany). The quickly mounted preparation was then immersed in the organ bath (20 ml) filled with KH solution. The KH solution (at 37°C, pH 7.4, gassed with 95% O₂/5% CO₂) was exchanged every 20 min. The force transducer was attached to micromanipulator which permitted displacement of the upper hook along a strict vertical axis and the adjustment of the muscle length. After a period of stabilization (60 min) the vascular muscle was stretched to its optimal length, which was established, in the preliminary experiments, to correspond to a counter-weight of 2 g (data not shown). The integrity of the endothelium in aortas was tested, at the end of the experiment, by pre-contracting the preparation with phenylephrine (0.1 µM) and, when the contraction reached a plateau, cumulative concentrations of the antidepressants were applied. In the last set of experiments preparations with endothelium were incubated for 30min with the potassium channel blocking agents charybdotoxine (0.05 µM, calcium dependent K⁺ channel), glibenclamide (10 µM, ATP-dependent K channel), tetrathyrammoniumchloride (TEA, 10 mM, non-specific K⁺ channel) or 4-aminopyridine (4-AP, 100 µM, voltage operated K⁺ channel).

Some endothelium intact preparations were pre-contracted with 40 mM KCl, then incubated for 30 minutes with propranolol (10 µM, β-adrenergic blocker) or prazosine (10 µM, α₁-adrenergic blocker) before cumulative concentrations of the antidepressants would be applied.
Amitriptyline hydrochloride, fluoxetine hydrochloride, tranylcypromine, venlafaxine hydrochloride, phenylephrine hydrochloride, 9-(tetrahydro-2-furanyl)-9H-purin-6-amine SQ 22536), N(G)-nitro-L-arginine methyl ester (L-NAME), 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one (ODQ), propranolol hydrochloride, prazosine hydrochloride, 4-aminopyridine, charybdotoxin, tetraethylammonium (all were dissolved in distilled water), or glibenclamide (which was dissolved in dimethylsulfoxide-DMSO) were purchased from Sigma-Aldrich, Deisenhofen, Germany; Thiopental (Trapanal®) was purchased from BYK, Konstanz, Germany. The maximal concentrations of the solvent DMSO that were used were inferior to 0.01%. When using high concentrations of KCl, NaCl concentration of the Krebs-Henseleit solution was reduced to achieve equimolarity.

Data analysis

Data are presented as means ±S.E.M. The concentrations of agonist that produced half-maximal effect (EC₅₀) were determined by means of non-linear regression using the Hill-Langmuir equation. The EC₅₀ was related to basic tension (before incubation) and only calculated from the positive values and given as positive numbers on the –log₁₀M, on the p scale as the pEC₅₀. Obtained data were compared using analysis of variance (one-way ANOVA) followed by Dunnet post-hoc test. Probabilities of less than 0.05 were considered statistically significant. Statistical analysis was performed using the software package Prism 4 (GraphPad Software, Inc., San Diego, CA).

RESULTS

Effects of incubation with antidepressants on phenylephrine induced contractions (Table 1)

Treatment with amitriptyline, fluoxetine shifted the concentration response curve of phenylephrine to the right in preparations compared to controls, incubation with tranylcypromine revealed a left shift (P<0.001). In preparations...
with endothelium higher concentrations of phenylephrine were required to contract the arteries than in endothelium free arteries (P<0.05).

**Effects of antidepressants on KCl pre-contracted arterial rings**

In KCl (20 mM) pre-contracted preparations, fluoxetine and amitriptyline induced complete relaxation which was concentration dependent (Fig. 1). Removal of endothelium did not have an effect. Cumulative concentrations of tranylcypromine hardly relaxed endothelium intact preparations, whereas venlafaxine did not have any effect to the endothelium intact nor endothelium denuded vessels. The pEC50 values could therefore not be calculated.

**Effects on phenylephrine pre-contracted arterial rings**

In phenylephrine (0.1 µM) pre-contracted preparations, cumulative concentrations amitriptyline, fluoxetine and tranylcypromine (in descending order of potency) induced complete relaxation of the arteries in a concentration dependent manner (Fig. 1). There were no differences between endothelium intact and endothelium free arteries. High doses of venlafaxine (>50 µM) induced further contraction of 110±23.2% from T_max in preparations with endothelium (the pEC50 value could therefore not be calculated), whereas endothelium free aortal rings were slightly dilated.

**Effects on prostaglandin F2α pre-contracted arterial rings**

In prostaglandin F2α (5 µM) pre-contracted arterial rings, none of the antidepressants lead to complete relaxation of arterial preparations with endothelium, while fluoxetine, amitriptyline and tranylcypromine relaxed those without endothelium (Fig. 1). Absence of endothelium apparently displaced the concentration-response curves to the left for fluoxetine dose responses (P<0.05). High concentrations of tranylcypromine and venlafaxine induced further contraction of preparations with endothelium (tranylcypromine >10 µM, 126±26% from T_max, venlafaxine >50 µM, 116±16 % from T_max). The pEC50 values could therefore not be calculated.

**Incubation experiments: effects of nitric oxide-cGMP or cAMP blockade**

The eNOS inhibitor L-NAME had already an influence on the basic arterial tone and led to a contraction of maximal 12% from T_max that was reached in pre-contraction by phenylephrine.
ODQ and SQ 22536 had a minor effect (maximal ±7% of T<sub>max</sub>) on the basic tone (Fig. 2). Incubation with L-NAME in phenylephrine (0.1 µM) pre-contracted preparations led to a right-shift of concentration-response curves for amitriptyline, fluoxetine and tranylcypromine (P<0.05). Also after pre-incubation with ODQ significant higher concentrations of fluoxetine and tranylcypromine were necessary to relax rat aorta (P<0.01). Incubation with L-NAME or ODQ had no effect on the dose response curve of venlafaxine, and the pEC<sub>50</sub> values could not be calculated. Incubation with SQ 22536 did not change dose response curves of any antidepressant examined.

**Incubation experiments: effects of potassium channel blockers**

TEA had a contracting effect (at the maximum 60% from T<sub>max</sub> reached by phenylephrine), similarly as charybdotoxin (maximal 11% of T<sub>max</sub>) or glibenclamide and 4-aminopyridin (max. ±7% of T<sub>max</sub>) (Fig. 2). Pre-incubation with 4-aminopyridin and TEA lead to a right shift of the relaxation response curves of fluoxetine and tranylcypromine with significant differences in the obtained pEC<sub>50</sub> values (P<0.05). Incubation with the K<sup>+</sup> channel blocking agents had no effect on the dose response curve of venlafaxine. Incubation with charybdotoxin or glibenclamide did not change the concentration response curves of any of the antidepressant investigated (data not shown).

**Effects of blockade of alpha-or beta-adrenoceptors**

Prazosine had no effect on concentration response curves of the antidepressants (results not shown). Propranolol incubation caused a left-shift for the concentration response curves of venlafaxine (control with endothelium 3.80±0.06, propranolol incubation 4.14±0.05, n=8; P<0.001). Propranolol did not change the dose-response curves of amitriptyline, fluoxetine or tranylcypromine.

**DISCUSSION**

In this study the principle mechanisms of smooth muscle contraction, namely the pharmacological and the electromechanical coupling (13, 14), are represented by the different pre-contraction conditions caused by phenylephrine, PGF<sub>2α</sub> or high extracellular potassium concentrations using KCl, respectively. Putative vasodilating substances can reduce the tone of arteries via inhibition of adrenal or prostaglandin receptors, thus interfering with the pharmacomechanical pathway, or, by an effect on electromechanical mechanism, for instance, by activating potassium channels.

The present in vitro experiments demonstrated that various classes of antidepressant agents had prominent direct vasoactive and predominantly vasodilatation properties on isometric tension in the elastic arteries of the rat and interact with the pharmacomechanical and the electromechanical mechanisms of contraction in vascular smooth muscle cells, as well. In addition, incubation with low concentrations of the antidepressants inhibited contracting adrenergic responses which are related to the NO-cGMP-pathway.

Our findings regarding fluoxetine are partially in agreement with previous findings. Ungvari et al. (15) also found no endothelium dependence of fluoxetine relaxing effects in their study performed on cerebellar arterioles. Nevertheless, in the present study low concentrations of fluoxetine inhibit vasoconstriction responses to adrenergic stimuli in a partially endothelium manner. Furthermore, inhibition of NO or cGMP production resulted in a decrease of the preparations’ sensitivity towards fluoxetine. It can be concluded that the NO-cGMP pathway is an important vasorelaxing mechanism of fluoxetine in the rat aorta. However, Ungvari et al. (15) described a fluoxetine induced inhibition of BAY K 8644 activated voltage gated calcium channels in cerebral vessels. It has to be verified whether fluoxetine interacts with calcium channels in aortal smooth muscle since these have been detected in rat aortal smooth muscle (16).

In the present finding, KC1 pre-contracted arteries could be rapidly diluted by cumulative concentrations of fluoxetine, indicating an interaction with the electromechanical coupling of this drug. The fact that inhibition of voltage dependent potassium channel reduces sensitivity of preparations to fluoxetine supports this finding.

It has been reported that venlafaxine is associated with induction or worsening of hypertension (8). It releases noradrenaline, serotonin and dopamine in the central nervous system (17). Furthermore, it has been shown that venlafaxine can potentiate noradrenaline-evoked venoconstriction of the dorsal hand vein (18) and may lead to a modest increase in pulmonary arterial pressure in the isolated perfused rat lung (19). Therefore, our finding that venlafaxine tends to contract endothelium intact rat aorta needs further clarification. Release of endothelium-derived contracting substances, i.e. endothelin-1, angiotensin II or prostaglandins, could be involved. The finding that propranolol incubation facilitates venlafaxine induced vasoconstriction may hint an interplay between venlafaxine action and β-adrenoceptors. However, since propranolol has been reported to act as an inhibitor to other cellular proteins, like protein kinase C (20), other intracellular mechanisms could be influenced by venlafaxine. It has been shown that venlafaxine interacts with the ATP dependent calcium uptake into the endoplasmatic reticulum, at least in neurons (21).

The vasoactive effects of amitriptyline have been extensively studied as it represents one typical tricyclic antidepressant. Our findings that amitriptyline relaxes aortal ring preparations rapidly after adrenergic elicited tension are in line with these previous reports and represent its α<sub>1</sub>-antagonistic property (5, 6, 22). Therefore, it relaxes arterial smooth muscle in an endothelium independent manner. The intense inhibition of α<sub>1</sub>-adrenergic induced vasoconstriction of the rat aorta is also well documented for other tricyclic antidepressants like nortriptyline and imipramine (23).

However, in the present study low concentrations of amitriptyline reveal an additional inhibition of adrenergic effects which is connected to the integrity of the endothelium. The finding that pre-treatment with the eNOS inhibitor L-NAME delays the relaxing effects of amitriptyline sustains this result. Tunçok et al. reported that L-NAME treatment of rats ameliorated amitriptyline effect on blood pressure arguing for an involvement of nitric oxide production (24). Furthermore, amitriptyline is an antagonist at postsynaptic cholinergic receptors and its anticholinergic effects in human smooth muscle cells has been described (25). Kalkan and co-workers (26) have extensively studied amitriptyline and its effects on adenosine receptors in rat isolated aorta. An additional vasorelaxing component due to interaction with these receptors should be taken into consideration.

Vasoactive properties of tranylcypromine have not been studied in detail, so far. In our study, it shows vasorelaxing properties only to a smaller extent, as compared to the other substances tested. It augments adrenergic effects on arterial smooth muscle contraction, presumably due to its mechanism of action as inhibitor of the monoaminoxidase resulting in an increasing concentration of noradrenaline. Tranylcypromine seems to interfere with the prostaglandin metabolism as it further contracts prostaglandin F<sub>2α</sub> pre-contracted aortal rings. This finding is of special interest, as tranylcypromine interferes with the liberation of arachidonic acid, the precursor of all prostanooids and inhibits the synthesis of prostacyclin (27). Some of the
'vascular' side effects of the agent may emerge from a deregulation in prostanoïd homeostasis. Recently, Bujak-Gizycka and co-workers (28) described the ability of rat aortic tissue to generate proangiotensinogen-12 as substrate for a renin-independent generation of angiotensinogen I and II. The latter play also a crucial role in local regulation of blood pressure homeostasis. Interference of the antidepressants with proangiotensinogen-12 and actions of the renin-angiotensinogen-system require further investigations. We used relatively high concentration of L-NAME which, although often used (29-31), may have yet effects to other enzymes and signaling pathways. Therefore the interpretation of these results must be taken with some reserve.

CONCLUSION

This study supports the hypothesis that antidepressant induced side effects on blood pressure are at least in part by their direct effect on blood vessels, since amitriptyline, fluoxetine and tranylcypromine showed vasorelaxing properties and venlafaxine further contracted aortal rings. These differences could be relevant when deciding whether to discontinue antidepressants or not before anaesthesia (32).

This study was performed on rat aorta, which is a conducting elastic blood vessel and not a resistance vessel and therefore not responsible for the modulation of blood pressure. It has to be proofed whether the antidepressant agents act similarly in small arteries and arterioles and it would be of clinical relevance to examine the effects of these drugs in human resistance arteries. Although the antidepressants relax vascular smooth muscle independently of the integrity of endothelium, used in low concentrations they change the contraction responses to adrenergic agents or not before anaesthesia (32).

Conflict of interests: None declared.

REFERENCES


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