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## Inhibitory effects of sertraline in rat isolated perfused kidneys and in isolated ring preparations of rat arteries

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### Abstract

**Objectives** Sertraline is often prescribed to patients suffering with end stage renal disease, but its action on kidney has not been investigated. We aimed to investigate the pharmacological action of sertraline on rat kidney with emphasis on the underlying mechanisms involved in the vascular actions of the drug.

**Methods** The effects of sertraline were evaluated in rat isolated perfused kidneys and on ring preparations of mesenteric or segmental rat renal artery.

**Key findings** In kidneys, sertraline prevented the effects of phenylephrine on perfusion pressure, glomerular filtration rate, urinary flow and renal vascular resistance. In mesenteric rings sertraline inhibited phenylephrine-induced contractions with potency 30-times lower than verapamil. Sertraline reversed sustained contractions induced by phenylephrine or 60 mM K<sup>+</sup> within a similar concentration range. In segmental isolated rings, sertraline also reversed contractions induced by phenylephrine or 60 mM K<sup>+</sup> with the same concentration range, but with higher potency compared with mesenteric preparations. Under Ca<sup>2+</sup>-free conditions, sertraline did not change the intracellularly-mediated phasic contractions induced by phenylephrine or caffeine. Sertraline was ineffective against contractions induced by extracellular Ca<sup>2+</sup> restoration after thapsigargin treatment and Ca<sup>2+</sup> store depletion with phenylephrine. Conversely, sertraline decreased the contractions induced by Ca<sup>2+</sup> addition in tissues under high K<sup>+</sup> solution or phenylephrine plus verapamil.

**Conclusions** In rat isolated kidneys and in rat ring preparations of mesenteric or renal vessels, sertraline had antispasmodic effects that appeared to be caused by a direct action on vascular smooth muscle cells. Its actions were ineffective against Ca<sup>2+</sup>-releasing intracellular pathways, but appeared to interfere with sarcolemmal Ca<sup>2+</sup> influx with reduced permeability of both receptor- and voltage-gated Ca<sup>2+</sup> channels.

**Keywords** antidepressant; mesenteric artery; renal function; segmental renal artery; selective serotonin reuptake inhibitors

### Introduction

The selective serotonin reuptake inhibitors are widely used in the treatment of several psychiatric diseases such as depression, panic disorder and obsessive-compulsive disorder.<sup>[1]</sup> Sertraline, in particular, is an antidepressant belonging to this drug class which is metabolized and excreted as an inactive compound, with a plasma half-life of approximately one day.<sup>[2]</sup> Renal dysfunctions are not mentioned as side effects in studies that established its efficacy and safety, but sertraline may be pharmacologically active on kidneys and blood vessels.<sup>[3–5]</sup> Such hypothesis is supported by previous studies showing that it was effective against hypotension caused by haemodialysis, probably through an improvement of the autonomic regulation in response to hypovolaemia.<sup>[6–8]</sup> Nevertheless, the establishment of its effects on kidneys may be of importance because this antidepressant is often prescribed to patients suffering with end stage renal disease, when depression is the most common psychological problem.<sup>[9]</sup>

Sertraline, like other serotonin reuptake inhibitors, has direct and indirect properties on smooth muscle cells, especially in vascular smooth muscle, ranging from vasorelaxant

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actions to potentiating effects on contractile responses induced by serotonin.<sup>[10–13]</sup> To the best of our knowledge, it is not yet known whether such vascular actions induced by sertraline also occurs in renal vessels, and whether they could be able to interfere with renal parameters such as perfusion pressure (PP) and glomerular filtration rate (GFR). In general, its inhibitory effects on vascular smooth muscle are often related to a putatively decreased  $\text{Ca}^{2+}$  entry through voltage-gated  $\text{Ca}^{2+}$  channels.<sup>[12]</sup> In this direction, we have investigated the pharmacological actions of sertraline on the functional parameters of rat isolated perfused kidneys, and on the responsiveness of isolated ring preparations of rat renal segmental or mesenteric arteries, with emphasis on the underlying mechanisms involved in its myogenic actions.

## Materials and Methods

### Animals

The study was performed on male Wistar albino rats (200–280 g) with approval from our Institutional Animal Ethics Committee (process 93/09). Animal welfare and experimental procedures were undertaken in accordance with the Ethical Principles for Care and Use of Laboratory Animals of the Brazilian Society for Laboratory Animal Science (<http://www.cobea.org.br>). Animals were housed in polypropylene cages in groups of six with 12 h dark/12 h light cycles at room temperature ranging from 22 to 26°C. Food and water were offered freely.

### Solutions and drugs

The perfusate consisted of a modified Krebs–Henseleit solution (MKHS (in mM): 118.0 NaCl, 1.2 KCl, 1.18  $\text{KH}_2\text{PO}_4$ , 1.18  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2.50  $\text{CaCl}_2$  and 25.0  $\text{NaHCO}_3$ ). Bovine serum albumin (BSA; 6 g) was added to 100 ml MKHS, and dialysed for 48 h at 4°C against 10 vol MKHS. In experiments with  $\text{Ca}^{2+}$ -free medium,  $\text{CaCl}_2$  was omitted from the normal MKHS and ethylene glycol-bis(b-aminoethyl ether)*N,N,N',N'*-tetraacetic acid (EGTA; 0.1–1 mM) was added. Sertraline was dissolved in ethoxydiglycol, brought to the chosen concentration with MKHS and vortexed just before use. In the bath chamber the highest concentration of ethoxydiglycol was 0.1% (v/v) and in such case it was without any measurable effects. Reagents were purchased from Sigma (St Louis, MO, USA) and dissolved directly in MKHS just before use. Sertraline was purchased from Galena Química e Farmacêutica Ltda. (Campinas, Brazil).

### Isolated perfused rat kidney

After anaesthesia with sodium pentobarbital (50 mg/kg, i.p.), the right renal artery was cannulated via the superior mesenteric artery without flow interruption to avoid ischaemic damage and placed into the perfusion line. Afterwards, the animals were killed by exsanguination. The perfusate solution was MKHS (final volume of 100 ml; pH 7.4) enriched with 100 mg glucose, 50 mg urea, and 50 mg inulin. The perfusion system had been described previously and consisted of an adaptation of the Bowman technique, employing a silastic membrane oxygenator in the perfusion line ( $\text{PO}_2$  at 60.0–66.7 kPa gassed with 5%  $\text{CO}_2$  in  $\text{O}_2$ ).<sup>[14,15]</sup> A period of 30 min

was allowed for equilibrium of the perfusion parameters and, once started, each experiment lasted 90 min. Samples of both urine and perfusate were collected at 10-min intervals for analysis. Inulin was determined in samples of the perfusate and urine by direct hydrolysis, as described previously.<sup>[14]</sup> Concentration–effect curves for phenylephrine were constructed by adding cumulative concentrations ( $10^{-11}$ – $10^{-4}$  M) to the perfusion system in the absence or in the presence of sertraline (30  $\mu\text{M}$ ) or vehicle (ethoxydiglycol 0.03% v/v). PP, renal vascular resistance, urinary flow and GFR were determined according to Martines-Maldonado *et al.*<sup>[16]</sup> In brief, PP was measured at the tip of a stainless steel cannula into the renal artery by a mercury manometer connected to the perfusion system. Urinary flow was determined by the ratio between the volume of urine collected at a given 10-min interval and the mass of the left kidney. Renal vascular resistance was measured by the ratio between PP and the renal perfusion flow obtained by a flow meter connected to the experimental system. GFR was determined by the inulin clearance.

### Mesenteric vessel assay

After the rats were killed by stunning and exsanguination, the major branch of mesenteric artery was rapidly removed and immersed in oxygenated MKHS. Fat and connective tissue were carefully removed, and ring segments ~3 mm in length were suspended by a pair of stainless-steel pins in an oxygenated (95%  $\text{O}_2$ –5%  $\text{CO}_2$ ) bath chamber containing 5 ml MKHS at 37°C. Force generation was recorded by isometric transducers (ML870B60/C-V, ADInstruments, Sydney, Australia) of a PowerLab 8/30 device. Resting tension was 0.5 g and preparations were equilibrated for 60 min. Control contractions were induced by adding 60 mM KCl until observation of two successive control contractions with similar amplitude to begin the experiments. To study the role of the endothelial layer on the effects induced by sertraline, either endothelium-intact or -denuded preparations were exposed to a given sertraline concentration for 10 min, and then 0.3  $\mu\text{M}$  phenylephrine was added to the bath still in the presence of sertraline. Endothelium removal was confirmed functionally by the relaxing effect of 1  $\mu\text{M}$  acetylcholine on 0.1  $\mu\text{M}$  phenylephrine-induced contractions. Some preparations were maintained under  $\text{Ca}^{2+}$ -free conditions and procedure details are given in the Results section. To compare the relaxing potency of sertraline in mesenteric or renal vessels, endothelium-intact rings of mesenteric artery (or segmental renal artery, see below) were initially exposed to phenylephrine (1  $\mu\text{M}$ ) or  $\text{K}^+$  (60 mM) and when the steady state of a given contraction was reached, sertraline (1–100  $\mu\text{M}$ ) was added cumulatively to construct a concentration–effect curve.

### Renal vessel assay

After the rats were killed by stunning and exsanguination, the first branches of renal artery (segmental artery) were removed, immersed immediately in oxygenated MKHS and dissected free of adhering tissue under a microscope. The rings were mounted in a 610 M-DMT Wire Myograph System (Danish Myo Technology A/S, Aarhus, Denmark). Two tungsten (40  $\mu\text{m}$ ) wires were passed through the lumen of the ring. One

of the wires was fixed to a micrometer for length adjustments, and the other was connected to a force transducer for isometric force measurements. Each ring was immersed in tissue baths (DMT, Denmark) containing MKHS, maintained at 37°C, and continuously bubbled with 95% O<sub>2</sub>–5% CO<sub>2</sub> (pH 7.4). Preparations were equilibrated for 1.5 h under a resting tension of 5 mN. The contractile capacity of each vessel ring was tested with 60 mM K<sup>+</sup> until observation of two reproducible contractions with similar amplitude to begin the experiments. After equilibration, segments were precontracted with 10 μM phenylephrine or 60 mM K<sup>+</sup>. Once the sustained tension was obtained, sertraline (0.3–100 μM) was added cumulatively to the bath and concentration–response curves were constructed. The endothelium integrity was confirmed functionally by the presence of a relaxing effect after 1 μM acetylcholine on the 60 mM K<sup>+</sup>-induced contraction.

### Statistical analysis

Data are means ± SEM with *n* representing the number of experiments. Maximal effects (E<sub>max</sub>) of phenylephrine, Ca<sup>2+</sup>, sertraline or verapamil were used to construct concentration–response curves, which were expressed as mg of contractile force, as a percentage of a given control contraction or as a percentage of the initial contraction induced by 60 mM K<sup>+</sup> as indicated. The significance (*P* < 0.05) of the results was assessed by unpaired Student's *t*-tests, one- or two-way analysis of variance followed by multiple comparison tests as indicated. IC<sub>50</sub> values, defined as the concentration of sertraline or verapamil that reduced the phenylephrine-induced contraction by 50%, was calculated by interpolation from semi-logarithmic plots, being expressed as geometric means (95% confidence interval; 95% CI).

## Results

### Effects of sertraline and phenylephrine on renal parameters of rat isolated kidneys

Kidney perfusion with sertraline (30 μM) alone did not change renal parameters (*P* > 0.05, analysis of variance), which remained unaltered throughout the entire evaluation period (90 min; data not shown). In contrast, perfusion with cumulative concentrations of phenylephrine (10<sup>-11</sup>–10<sup>-4</sup> M, *n* = 4; Figure 1) increased significantly (*P* < 0.05, Dunn test) urinary flow from its control values of 0.11 ± 0.02 to 0.43 ± 0.15 ml/g/min in the presence of phenylephrine 10<sup>-4</sup> M. At this concentration, phenylephrine also significantly (*P* < 0.05, Dunn test) increased the values of PP from 108.00 ± 0.78 to 178.31 ± 21.68 mmHg, while renal vascular resistance was increased from 4.09 ± 0.08 mmHg/ml/g/min in the control period to 6.81 ± 0.99 mmHg/ml/g/min. Phenylephrine also significantly increased the values of GFR to 1.60 ± 0.40 ml/g/min (vs 0.44 ± 0.08 ml/g/min in the absence of phenylephrine; *P* < 0.05, Dunn test). When the isolated kidneys had been previously perfused (for 10 min) with sertraline (30 μM, *n* = 4), phenylephrine was ineffective in inducing functional changes, even at the highest concentration used in this study (10<sup>-4</sup> M). Under such conditions (at 10<sup>-4</sup> M phenylephrine), the values of renal parameters were: urinary flow = 0.08 ± 0.03 ml/g/min, PP = 105.39 ± 14.46 mmHg, renal vascular

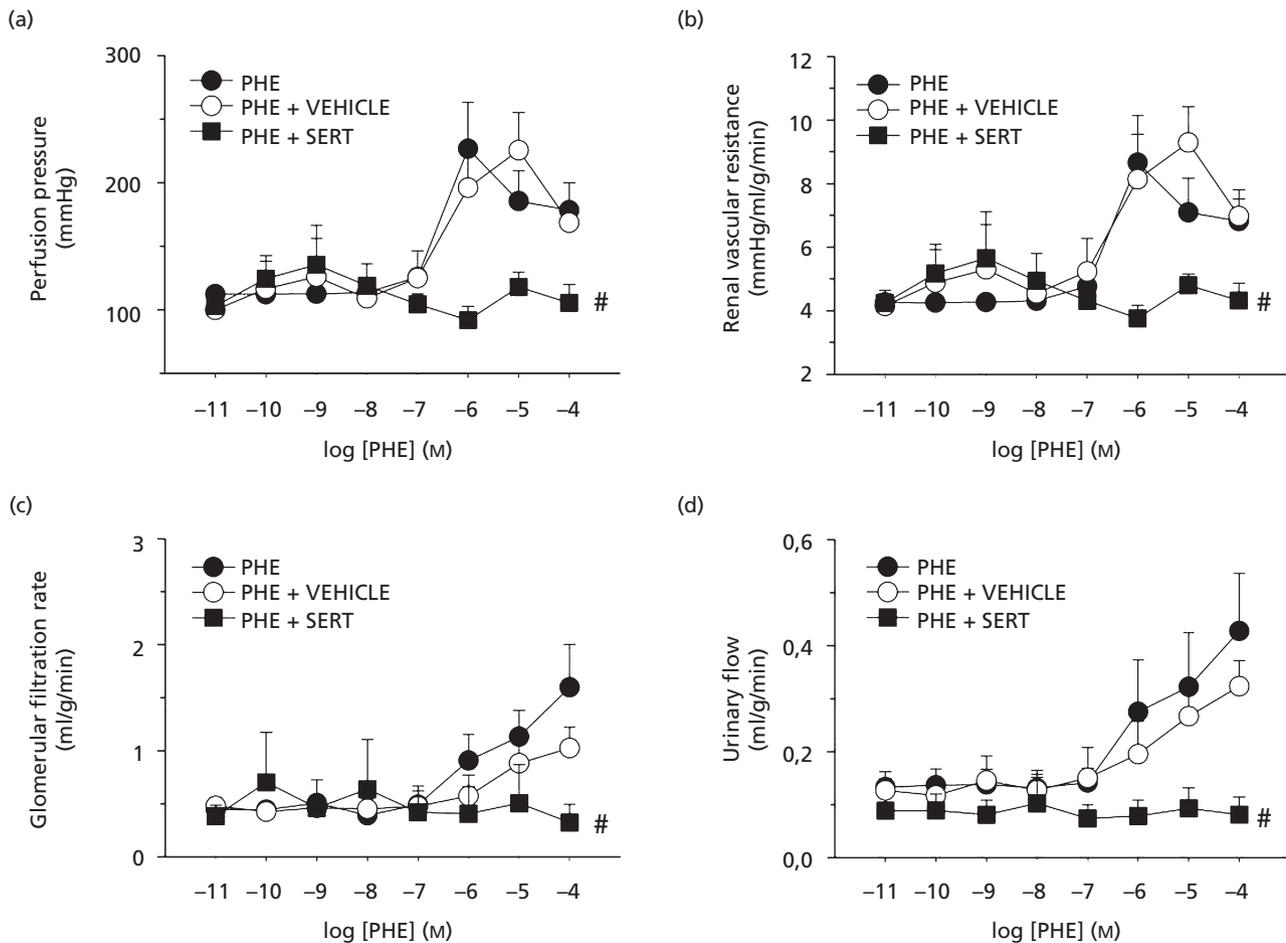
resistance = 4.32 ± 0.55 mmHg/ml/g/min and GFR = 0.32 ± 0.17 ml/g/min, which did not differ from the values recorded in kidneys perfused under control conditions (*P* > 0.05, analysis of variance). Vehicle alone did not alter the phenylephrine-induced effects on renal parameters.

### Relaxant effects of sertraline on the sustained contractions induced by phenylephrine or K<sup>+</sup> on ring preparations of segmental renal or mesenteric artery

Endothelium-intact rings of segmental artery were firstly exposed to phenylephrine (10 μM) or K<sup>+</sup> (60 mM) and when the steady state of a given contraction was reached, sertraline (0.3–100 μM) was cumulatively added to construct a concentration–effect curve. Under such conditions, sertraline significantly (*P* < 0.05, Holm-Sidak test) relaxed the contractions induced by phenylephrine (8.7 ± 2.4 mN; *n* = 7) with IC<sub>50</sub> values of 4.7 (95% CI, 2.9 to 7.7) μM (Figure 2a). At 30 μM, sertraline reduced the phenylephrine-induced contraction to 6.3 ± 1.6% of the contraction in its absence and a virtually complete inhibition was observed at 60 μM (1.4 ± 0.8% of the control contraction). In another set of experiments, sertraline (0.3–100 μM) significantly (*P* < 0.05, Holm-Sidak test) relaxed the sustained contractions induced by 60 mM K<sup>+</sup> (5.8 ± 1.5 mN; *n* = 6) in a concentration-dependent manner. The IC<sub>50</sub> values were 7.7 (95% CI, 3.7 to 16.1) μM with the remaining contractile response in presence of 60 μM sertraline corresponding to 6.0 ± 2.9% of the control contraction. The statistical comparison between IC<sub>50</sub> values for phenylephrine and K<sup>+</sup> showed no significant difference (*P* > 0.05, Student's *t*-test).

Endothelium-intact mesenteric rings were initially exposed to phenylephrine (1 μM) or K<sup>+</sup> (60 mM) and when the steady state of a given contraction was reached, sertraline (1–100 μM) was cumulatively added to construct a concentration–effect curve (Figure 2b). Under such conditions, sertraline induced a relaxing effect with IC<sub>50</sub> values of 16.9 (95% CI, 10.2 to 27.8) μM (*n* = 11) and 23.3 (95% CI, 12.7 to 42.7) μM (*n* = 8) to phenylephrine- and K<sup>+</sup>-induced contractions, respectively, values that did not reach statistical significance (*P* > 0.05, Student's *t*-test). However, IC<sub>50</sub> values were significantly higher in mesenteric than in segmental renal artery to a given contractile agent (*P* < 0.05, Student's *t*-test). In addition, at 30 μM, sertraline reduced the phenylephrine-induced contraction to 38.6 ± 4.1% of the control contraction, values significantly higher than those obtained in segmental artery under similar conditions (*P* < 0.05, Student's *t*-test). A virtually complete inhibition was not observed even at 100 μM sertraline as shown in Figure 2b.

Additional experiments were performed using the α<sub>1</sub>-antagonist prazosin in mesenteric rings exposed to phenylephrine (1 μM) or K<sup>+</sup> (60 mM). After reaching the steady state of a given contraction, prazosin (0.01–10 μM) was cumulatively added to construct a concentration–effect curve (Figure 2c). Then, prazosin induced a relaxing effect on phenylephrine-induced contraction with IC<sub>50</sub> values of 0.06 (95% CI, 0.02 to 0.16) μM (*n* = 4) and full relaxation at 3 μM. However, K<sup>+</sup>-induced contraction was not changed by treatment with prazosin.



**Figure 1** Inhibitory effects of sertraline on phenylephrine-induced actions in rat isolated kidneys. The changes in renal parameters of rat isolated kidneys after perfusion with phenylephrine (PHE;  $10^{-11}$ – $10^{-4}$  M;  $n = 4$ ) in the absence or in the continuous presence of sertraline (SERT;  $30 \mu\text{M}$ ;  $n = 4$ ). The renal parameters were evaluated at 10-min intervals under a given phenylephrine concentration. In a separate group, kidneys were perfused only with the vehicle (ethoxydiglycol, 0.03% v/v). #The comparison en bloc between the raw data of isolated kidneys treated with sertraline and those treated with phenylephrine alone reached a significant difference ( $P < 0.05$ , two-way analysis of variance).

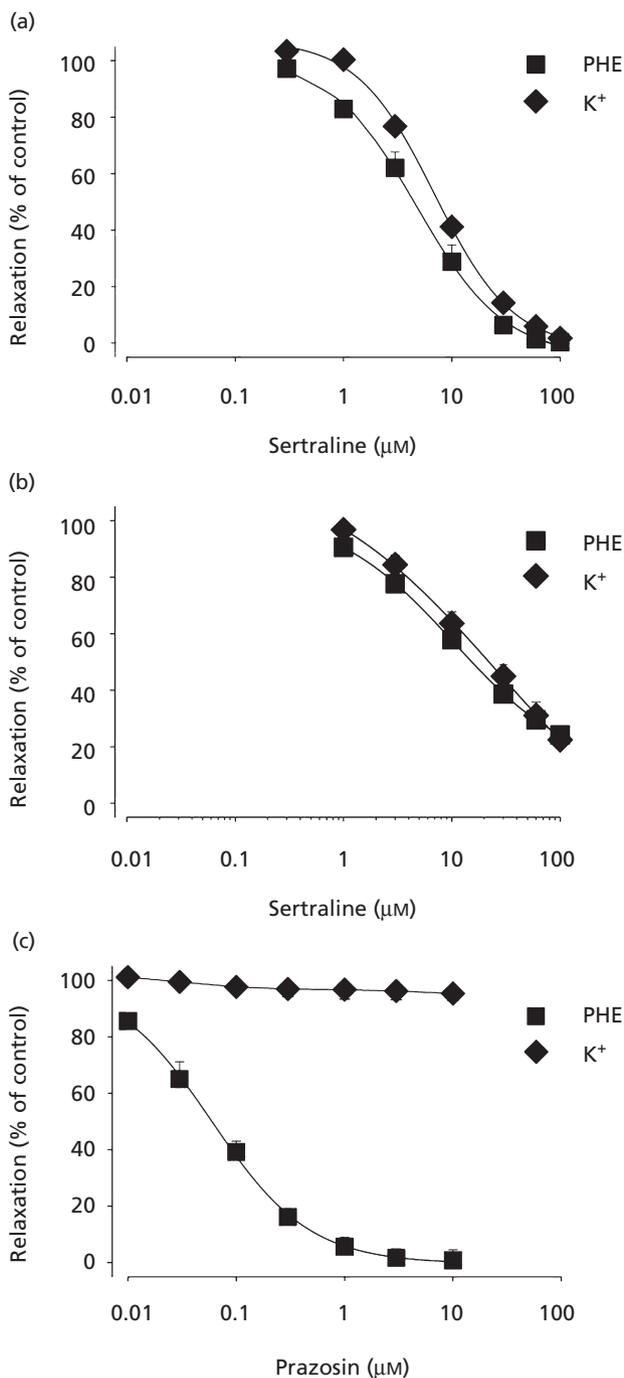
**Effect of endothelium removal on the inhibitory effects of sertraline on isolated mesenteric vessels**

In isolated endothelium-intact mesenteric rings, sertraline ( $1$ – $100 \mu\text{M}$ ), previously added to the contractile agent for 10 min, significantly ( $P < 0.05$ , Holm-Sidak test) inhibited the contractions induced by a submaximal concentration of phenylephrine ( $0.3 \mu\text{M}$ ;  $320.7 \pm 58.2$  mg of force contraction;  $n = 5$ ) in a concentration-dependent manner (Figure 3). The  $\text{IC}_{50}$  value for sertraline-induced inhibition was  $15.1$  (95% CI,  $9.2$  to  $24.6$ )  $\mu\text{M}$  ( $n = 5$ ) and a virtually complete inhibition was observed at  $100 \mu\text{M}$  ( $4.1 \pm 0.8\%$  of the control contraction). In endothelium-denuded preparations, sertraline caused nearly full inhibition of the phenylephrine-induced contraction ( $401.8 \pm 51.2$  mg;  $n = 6$ ), but with an increased value of  $\text{IC}_{50}$ , which corresponded to  $28.8$  (95% CI,  $20.4$  to  $40.6$ )  $\mu\text{M}$  ( $n = 6$ ;  $P < 0.05$ , Student's  $t$ -test). In endothelium-intact rings, verapamil decreased phenylephrine-elicited contraction with an  $\text{IC}_{50}$  value of  $0.5$  (95% CI,  $0.2$  to  $1.3$ )  $\mu\text{M}$  ( $n = 5$ ), values

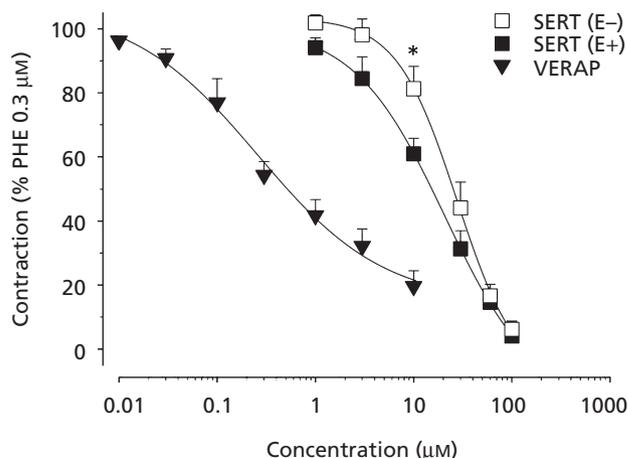
significantly smaller than those observed for sertraline ( $P < 0.05$ , Student's  $t$ -test).

**Effects of sertraline on phasic contractions of isolated mesenteric rings maintained under  $\text{Ca}^{2+}$ -free conditions**

Sertraline had no inhibitory effect on the phasic contractions induced by phenylephrine, caffeine or  $\text{Ca}^{2+}$  restoration under  $\text{Ca}^{2+}$ -free conditions on mesenteric rings. Some mesenteric rings were submitted to  $\text{Ca}^{2+}$ -free conditions (MKHS without  $\text{CaCl}_2$  containing  $0.1 \text{ mM}$  EGTA). Under such conditions, phenylephrine ( $1 \mu\text{M}$ ) induced a phasic contraction ( $117.3 \pm 24.3$  mg;  $n = 10$ ; Figure 4a) significantly smaller ( $P < 0.05$ , Student's  $t$ -test) than those obtained with phenylephrine in medium containing  $2.5 \text{ mM}$   $\text{Ca}^{2+}$  ( $460.9 \pm 82.3$  mg;  $n = 6$ ). In the presence of sertraline ( $100 \mu\text{M}$ ), this phasic contraction reached  $88.8 \pm 25.1$  mg ( $n = 9$ ), without significant difference compared with contractions in the absence of sertraline ( $P > 0.05$ , Student's  $t$ -test). When, under  $\text{Ca}^{2+}$ -free conditions,



**Figure 2** Relaxant effects of sertraline on sustained contractions induced by phenylephrine or  $K^+$  on ring preparations of rat segmental renal or mesenteric artery. The inhibitory effects of sertraline added on the steady state of a sustained contraction induced by phenylephrine (PHE; a, 10  $\mu$ M; b and c, 1  $\mu$ M) or  $K^+$  (60 mM) in isolated ring preparations of segmental renal (a, sertraline 0.3–100  $\mu$ M;  $n = 6-7$ ) or mesenteric (b, sertraline 1–100  $\mu$ M;  $n = 8-11$ ) artery. The experiments were performed in endothelium-intact preparations. (c) The relaxing effect induced by prazosin (0.01–10  $\mu$ M) in ring preparations of mesenteric vessels. Values are shown as % of a given contraction before addition of sertraline or prazosin.

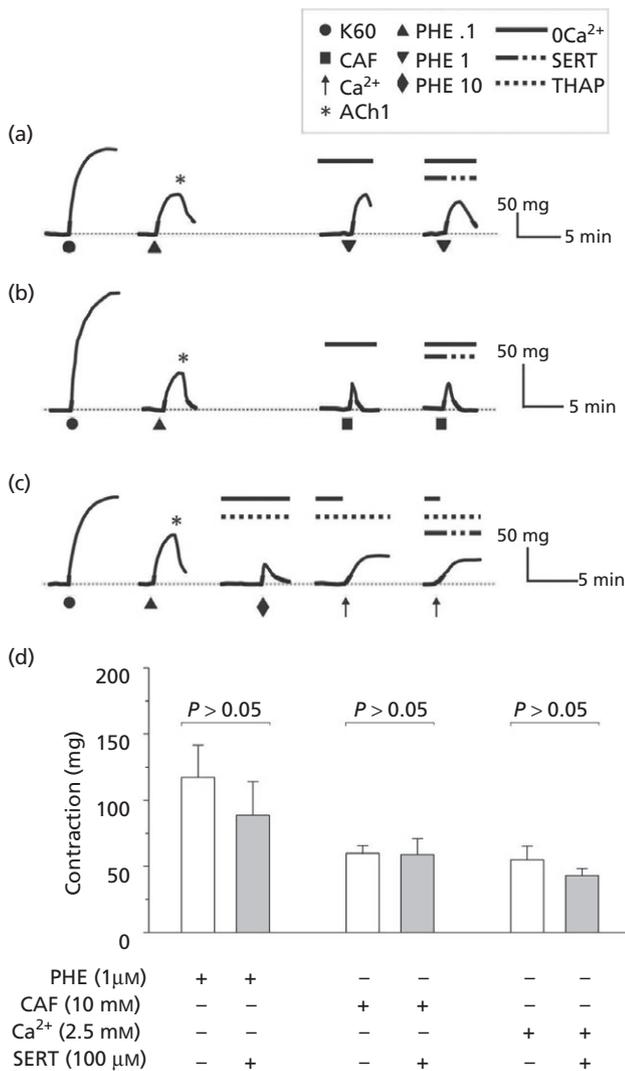


**Figure 3** Effects of endothelium removal on the inhibitory effects of sertraline on phenylephrine-induced contractions in mesenteric rings. The inhibitory effects of sertraline (SERT; 1–100  $\mu$ M) or verapamil (VERAP; 0.01–10  $\mu$ M) on the contractions induced by a submaximal concentration of phenylephrine (PHE; 0.3  $\mu$ M) in isolated mesenteric rings. The experiments with sertraline were performed in preparations containing functional endothelium (E+;  $n = 5$ ) or not (E-;  $n = 6$ ), whereas verapamil was used as positive control just in endothelium-intact preparations ( $n = 5$ ). \*The inhibition induced by sertraline 10  $\mu$ M was significantly smaller in endothelium-denuded than in endothelium-intact rings ( $P < 0.05$ , two-way analysis of variance followed by Holm-Sidak test).

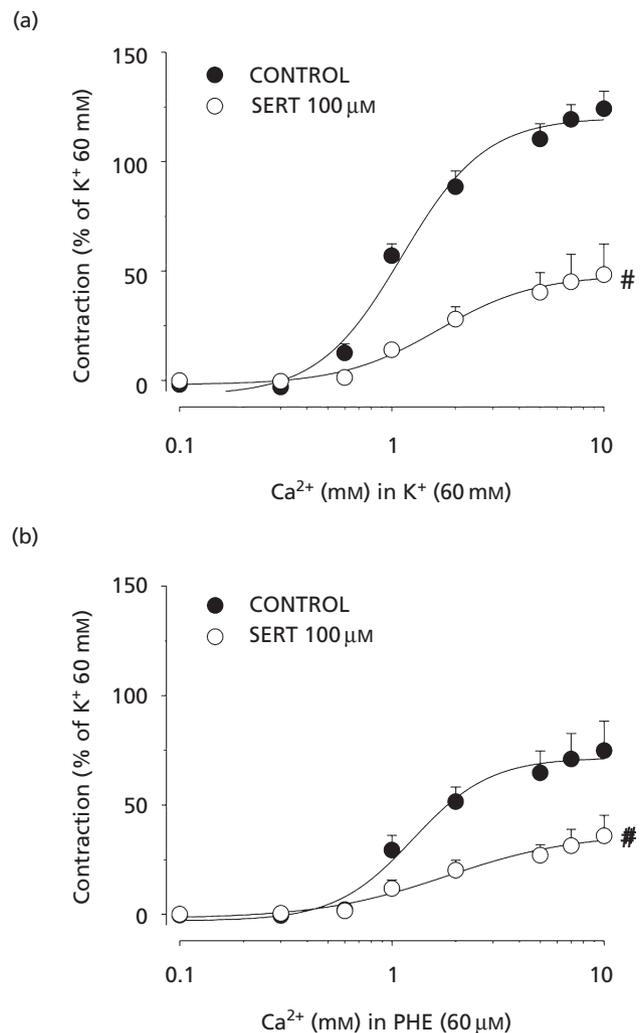
the contractile stimulus was caffeine (10 mM, at 25°C; Figure 4b) instead of phenylephrine, mesenteric rings produced a transient contraction of  $59.8 \pm 5.9$  mg ( $n = 6$ ) in the absence and  $59.0 \pm 12.2$  mg ( $n = 8$ ) in the presence of sertraline (100  $\mu$ M). These values did not differ significantly ( $P > 0.05$ , Student's  $t$ -test). Additionally, a group of mesenteric rings maintained in  $Ca^{2+}$ -free medium containing 1 mM EGTA and 0.1  $\mu$ M thapsigargin was stimulated with phenylephrine (10  $\mu$ M) to deplete intracellular  $Ca^{2+}$  stores (Figure 4c). After phenylephrine removal, the normal concentration of 2.5 mM  $Ca^{2+}$  was then restored and produced a contraction of  $54.9 \pm 10.3$  mg ( $n = 5$ ) in the absence and  $42.9 \pm 5.5$  mg ( $n = 5$ ) in the presence of sertraline (100  $\mu$ M). Once again, these values did not reach statistical significance ( $P > 0.05$ , Student's  $t$ -test).

**Effects of sertraline on the concentration–effect curves induced by  $Ca^{2+}$  addition in mesenteric rings maintained in presence of verapamil and phenylephrine or 60 mM  $K^+$**

A group of mesenteric rings was maintained under  $Ca^{2+}$ -free conditions (MKHS with 0.5 mM EGTA) and was stimulated with either a high concentration of phenylephrine (60  $\mu$ M) in the presence of verapamil (3  $\mu$ M; Figure 5b) or with a high concentration of  $K^+$  (60 mM; Figure 5a). (The concentration of verapamil was chosen because it blocked completely  $K^+$ -induced contractions in mesenteric rings (data not shown).) Under such conditions, mesenteric rings produced unsustained contractions that rapidly returned to baseline and, then, a concentration–effect curve for  $Ca^{2+}$  (0.1–10 mM; Figure 5) was constructed in the absence or in the presence of sertraline. Compared with the initial contractions induced by



**Figure 4** Effects of sertraline on phasic contractions of isolated mesenteric rings maintained under Ca<sup>2+</sup>-free conditions. (a)–(c) Trace recordings of typical experiments with an initial contraction induced by 60 mM K<sup>+</sup> that served as a reference in Ca<sup>2+</sup>-containing medium. To test the endothelial integrity, the rings were challenged to contract with phenylephrine (PHE; ▲; 0.1 μM) and acetylcholine (\*; 1 μM) was added on the plateau of the phenylephrine-induced contraction. The relaxation induced afterwards indicated endothelium functionality. The mesenteric rings were submitted to Ca<sup>2+</sup>-free conditions (modified Krebs–Henseleit solution without Ca<sup>2+</sup> with 0.1 mM EGTA). After 6 min under Ca<sup>2+</sup>-free conditions, they were stimulated by phenylephrine (a: ▼; 1 μM; n = 9–10) or caffeine (b: CAF; ■; 10 mM at 25°C; n = 6–8) in the absence or in the presence of sertraline (SERT; 100 μM). (c) Mesenteric rings were treated in Ca<sup>2+</sup>-free conditions with EGTA (1 mM) and thapsigargin (THAP; 0.1 μM) being subsequently stimulated with phenylephrine (◆; 10 μM) to deplete the intracellular Ca<sup>2+</sup> stores. After phenylephrine removal, they contracted with the addition of Ca<sup>2+</sup> (†; 2.5 mM with removal of EGTA; n = 5) either in the absence or in the presence of sertraline. (d) The mean values of these contractions, showing that sertraline was unable to induce inhibitory changes in effects produced by phenylephrine, caffeine, or Ca<sup>2+</sup> (P > 0.05, unpaired Student’s *t*-test). Crosses below the graph indicate treatments.



**Figure 5** Effects of sertraline on the concentration–effect induced by Ca<sup>2+</sup> on mesenteric rings in presence of either K<sup>+</sup> or phenylephrine in presence of verapamil. The concentration-dependent contractions induced by Ca<sup>2+</sup> (0.1–10 mM) in mesenteric rings maintained in Ca<sup>2+</sup>-free medium (with 0.5 mM EGTA). Before Ca<sup>2+</sup> addition, mesenteric rings were treated and maintained in solutions containing either (a) 60 mM K<sup>+</sup> (n = 5) or (b) phenylephrine (60 μM) plus verapamil (3 μM) (n = 6). #The comparison en bloc of the concentration–effect curve between mesenteric rings maintained in the absence (control) or in the presence of sertraline (100 μM) reached a significant difference (P < 0.05, two-way analysis of variance).

60 mM K<sup>+</sup>, which served as reference, E<sub>max</sub> values were obtained at 10 mM Ca<sup>2+</sup> and corresponded to 124.1 ± 8.1% (n = 5) and 74.8 ± 13.5% (n = 6) in rings stimulated with K<sup>+</sup> or phenylephrine, respectively. In the presence of sertraline (100 μM) these values were significantly reduced (P < 0.05, two-way analysis of variance) to 48.3 ± 14.0% and 35.9 ± 9.4% in K<sup>+</sup>- and phenylephrine-stimulated preparations, respectively.

### Discussion

This study has shown that the perfusion of rat isolated kidneys with a single concentration of sertraline impaired the changes

promoted by phenylephrine in several renal parameters. Such effect appears to be mainly due to the inhibitory action of sertraline against the contractile effects induced by this adrenergic agonist on renal vasculature. In fact, sertraline showed direct antispasmodic effects on rat isolated mesenteric artery by a putatively diminished  $\text{Ca}^{2+}$  entry from the extracellular milieu.

Under standard conditions, the continuous perfusion of the rat kidneys with sertraline did not change the renal parameters, which remained stable throughout the experimental period, repeating similar results observed with vehicle (ethoxydiglycol)-perfused kidneys (data not shown) and confirming the reliability of the isolated kidney preparation used herein. On the other hand, when kidneys were perfused with increasing concentrations of phenylephrine, all renal parameters were augmented by virtue of the phenylephrine-induced activation of  $\alpha_1$ -adrenoceptors. Once activated by the presence of catecholamines,  $\alpha_1$  receptors increase vascular resistance as a result of renal vasoconstriction, which is part of the underlying physiological mechanisms that regulate renal haemodynamics.<sup>[17–19]</sup>

Interestingly, when sertraline was previously added into the perfusion solution, the effects induced by phenylephrine were prevented, revealing an inhibitory property of this antidepressant agent in renal vessels. In fact, a few studies have already reported that sertraline has inhibitory effects in human blood vessels such as mesenteric and internal mammary arteries, as well as in rat aortic rings.<sup>[11,13]</sup> Smooth muscle relaxation was also described as a pharmacological property of other antidepressants belonging to the pharmacological family of the serotonin reuptake inhibitors.<sup>[20,21]</sup> Here, the experiments using rat isolated mesenteric artery confirmed that sertraline has vascular antispasmodic effects since it inhibited the contractions induced by phenylephrine. Compared with the positive control verapamil, sertraline showed a lower pharmacological potency since its  $\text{IC}_{50}$  values were approximately 30-times higher than those observed with verapamil (0.5  $\mu\text{M}$  for verapamil vs 15  $\mu\text{M}$  for sertraline).

It is noteworthy that, in perfused kidneys, sertraline abolished the renal responses to phenylephrine, whereas at 30  $\mu\text{M}$  it reduced just partially (between 31 and 38% of the control response) the phenylephrine-induced contractions in mesenteric rings, indicating that in renal vasculature sertraline may have exerted a more effective inhibition of phenylephrine than in other vascular beds. In fact, sertraline showed a higher potency in renal segmental artery than in mesenteric vessels. Notwithstanding, such effects occurred in a concentration range higher than that expected to be reached in plasma under clinical conditions.<sup>[21]</sup> However, it should be considered that it was similar to those previously described in either rat aorta or human blood vessels.<sup>[11–13]</sup> It is well known that plasma concentrations for most antidepressants are not yet securely established and factors such as the volume of distribution interfere with their plasma levels.<sup>[22]</sup> In general, sertraline is relatively lipophilic which may produce extensive distribution into tissues. As a matter of fact, Tremaine *et al.*<sup>[23]</sup> reported that the whole brain concentration of sertraline in the rat was more than 40-fold higher than that in plasma and its volume of distribution was approximately 25 l/kg.

The antispasmodic effects of sertraline were slightly dependent of the endothelium integrity, revealing that it exerted its major vasoactive actions by endothelium-independent pathways. At least in mice corpus cavernosum, but not in rat aorta, the release of nitric oxide was already related as a probable mechanism involved in the relaxant effects of sertraline.<sup>[13,24]</sup> Thus, it was reasonable to consider that sertraline exerted peripheral actions at higher concentrations than those necessary to obtain centrally-mediated effects as reported by Seo *et al.*<sup>[25]</sup>, probably because the underlying mechanisms involved in its vascular actions were different to those involved in the 5-hydroxytryptamine reuptake inhibition.

To further study how sertraline decreased vascular responsiveness to phenylephrine, additional experiments were performed using the antidepressant at 100  $\mu\text{M}$ , which caused pronounced inhibitory effects on the adrenergic response in mesenteric rings. On smooth muscle cells, the G protein-coupled  $\alpha_1$ -adrenoceptors mediated vascular contraction increasing intracellular  $\text{Ca}^{2+}$  levels ( $[\text{Ca}^{2+}]_i$ ).<sup>[26]</sup> Once activated, they triggered the phospholipase C-induced formation of the second messenger inositol triphosphate ( $\text{IP}_3$ ) and diacylglycerol.<sup>[27]</sup>  $\text{IP}_3$  can rapidly mobilize  $\text{Ca}^{2+}$  from its intracellular stores such as the sarcoplasmic reticulum causing a transient increase in the  $[\text{Ca}^{2+}]_i$ , which could be associated with the phasic (unsustained) contraction induced by phenylephrine in mesenteric rings under  $\text{Ca}^{2+}$ -free medium. Since sertraline did not change the phasic contraction induced by phenylephrine under such conditions, we concluded that it was unlikely that its inhibitory effects on mesenteric rings were mediated by interference in the  $\text{IP}_3$  ability to promote intracellular  $\text{Ca}^{2+}$  release.

Similarly, the experiments performed under  $\text{Ca}^{2+}$ -free conditions with either caffeine or with extracellular  $\text{Ca}^{2+}$  restoration after thapsigargin treatment allowed us to conclude that it was unlikely that sertraline acted by means of interference with two other cellular pathways related to  $[\text{Ca}^{2+}]_i$  regulation, i.e. the  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release and the capacitance  $\text{Ca}^{2+}$  entry, respectively. Caffeine induces  $\text{Ca}^{2+}$  release from the caffeine-releasable ryanodine-sensitive  $\text{Ca}^{2+}$  pool in smooth muscle sarcoplasmic reticulum, whereas smooth muscle cells depleted of its intracellular  $\text{Ca}^{2+}$  stores by a combination between the sarco/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase inhibitor thapsigargin and phenylephrine activate the so-called store-gated channels.<sup>[28,29]</sup> So, sertraline probably inhibited mesenteric contractions by changing  $\text{Ca}^{2+}$  entry by other pathways.

In general, the underlying mechanisms involved in the inhibitory effects of the antidepressants on vascular smooth muscle contractions are not yet entirely elucidated, but their ability to inhibit transmembrane  $\text{Ca}^{2+}$  entry are commonly reported, especially through the voltage-dependent  $\text{Ca}^{2+}$  channels.<sup>[12,20]</sup> Under  $\text{Ca}^{2+}$ -free conditions, sertraline diminished the contractions induced by  $\text{Ca}^{2+}$  addition in mesenteric rings maintained in  $\text{K}^+$  enriched (60 mM) medium. Once depolarized by the high  $\text{K}^+$  content in extracellular solution, the smooth muscle cell promotes opening of voltage-gated  $\text{Ca}^{2+}$  channels and this process allows  $\text{Ca}^{2+}$  to enter the cell down its electrochemical gradient.<sup>[30]</sup> Thus, according to our data using preparations depolarized with high  $\text{K}^+$  solutions: it was unlikely that a direct antagonism of sertraline on the

$\alpha$ -adrenergic receptor may have occurred; and our results corroborated the general concept that sertraline was able to decrease  $\text{Ca}^{2+}$  entry through L-type voltage-dependent  $\text{Ca}^{2+}$  channels, as suggested in either vascular or nonvascular tissues.<sup>[12,13,31]</sup>

A putative direct interaction with  $\alpha$ -adrenergic receptors could not convincingly explain the present effects of sertraline, because the relaxing effects on the sustained contraction induced by phenylephrine or by a high concentration of  $\text{K}^+$  showed a similar concentration range and  $\text{IC}_{50}$  values that did not differ significantly, revealing that its inhibitory actions were not specific to  $\alpha$ -adrenergic-elicited mechanisms. Such a conclusion was reinforced by the findings obtained with the typical  $\alpha_1$ -adrenoceptor blocker prazosin, which relaxed only the phenylephrine-induced contraction, while it was unable to reverse the contraction induced electromechanically by  $\text{K}^+$ . Molin and Bendhack<sup>[32]</sup> reported that the  $\alpha_2$ -agonist clonidine was able to relax phenylephrine-induced contraction but it was inert against  $\text{K}^+$ -elicited contraction. Thus, it was unlikely that sertraline behaved as an anti-adrenergic agent in rat vessels.

In mesenteric preparations maintained under  $\text{Ca}^{2+}$ -free medium containing phenylephrine and L-type channel blocker verapamil, which was used at a concentration that fully blocked the response induced by the high  $\text{K}^+$ -induced electromechanical coupling (data not shown), an inhibitory effect was also observed when sertraline was added previously to  $\text{Ca}^{2+}$  addition. Under such conditions and in response to  $\alpha_1$ -adrenergic receptor activation with phenylephrine, smooth muscle cells may have activated verapamil-resistant  $\text{Ca}^{2+}$  entry by a receptor-gated pathway, i.e. the pharmacomechanical coupling, which was triggered by a high concentration of phenylephrine (60  $\mu\text{M}$ ).<sup>[33]</sup> This profile of action for sertraline involving putatively inhibited receptor-gated  $\text{Ca}^{2+}$  channels has not been previously described.

## Conclusions

Taken as a whole, the effects induced by sertraline in rat isolated kidneys were consistent with an antispasmodic action on blood vessels. Such antispasmodic action was confirmed in isolated rings of mesenteric and segmental renal arteries and it appeared to be caused by a direct action of this substance on vascular smooth muscle cells. Its actions did not appear to occur by inhibition of  $\text{Ca}^{2+}$ -releasing intracellular pathways, but through the interference with the plasmalemmal  $\text{Ca}^{2+}$  influx reducing the permeability of both receptor- and voltage-gated  $\text{Ca}^{2+}$  channels. Our results were consistent with other findings reported previously to sertraline and to other antidepressants belonging to the selective serotonin reuptake inhibitors. Notwithstanding, definitive demonstration of the sertraline ability in reducing plasmalemmal  $\text{Ca}^{2+}$  currents deserves further investigation.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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## References

1. Muijsers RB *et al.* Sertraline: a review of its use in the management of major depressive disorder in elderly patients. *Drugs Aging* 2002; 19: 377–392.
2. Preskorn SH. Clinically relevant pharmacology of selective serotonin reuptake inhibitors. An overview with emphasis on pharmacokinetics and effects on oxidative drug metabolism. *Clin Pharmacokinet* 1997; 32 (Suppl. 1): 1–21.
3. Finkel SI *et al.* A randomized, placebo-controlled study of the efficacy and safety of sertraline in the treatment of the behavioral manifestations of Alzheimer's disease in outpatients treated with donepezil. *Int J Geriatr Psychiatry* 2004; 19: 9–18.
4. Löwe B *et al.* Efficacy, predictors of therapy response, and safety of sertraline in routine clinical practice: prospective, open-label, non-interventional postmarketing surveillance study in 1878 patients. *J Affect Disord* 2005; 87: 271–279.
5. Torta R *et al.* Sertraline effectiveness and safety in depressed oncological patients. *Support Care Cancer* 2008; 16: 83–91.
6. Dheenan S *et al.* Effect of sertraline hydrochloride on dialysis hypotension. *Am J Kidney Dis* 1998; 31: 624–630.
7. Perazella MA. Pharmacologic options available to treat symptomatic intradialytic hypotension. *Am J Kidney Dis* 2001; 38: (4 Suppl. 4): S26–S36.
8. Yalcin AU *et al.* Effect of sertraline hydrochloride on cardiac autonomic dysfunction in patients with hemodialysis-induced hypotension. *Nephron Physiol* 2003; 93: P21–P28.
9. Wuerth D *et al.* Identification and treatment of depression in a cohort of patients maintained on chronic peritoneal dialysis. *Am J Kidney Dis* 2001; 37: 1011–1017.
10. Gruetter CA *et al.* Potentiation of 5-hydroxytryptamine-induced contraction in rat aorta by chlorpheniramine, citalopram and fluoxetine. *Eur J Pharmacol* 1992; 217: 109–118.
11. Vila JM *et al.* Relaxant effects of antidepressants on human isolated mesenteric arteries. *Br J Clin Pharmacol* 1999; 48: 223–229.
12. Becker B *et al.* Blockade of calcium entry in smooth muscle cells by the antidepressant imipramine. *Biochem Pharmacol* 2004; 68: 833–842.
13. van Melle JP *et al.* Sertraline causes strong coronary vasodilation: possible relevance for cardioprotection by selective serotonin reuptake inhibitors. *Cardiovasc Drugs Ther* 2004; 18: 441–447.
14. Fonteles MC *et al.* Support of kidney function by long-chain fatty acids derived from renal tissue. *Am J Physiol* 1983; 244: F235–F246.
15. Bowman RH. Gluconeogenesis in the isolated perfused rat kidney. *J Biol Chem* 1970; 245: 1604–1612.
16. Martines-Maldonado M *et al.* Renal effects of lithium administration in rats: alterations in water and electrolyte metabolism and the response to vasopressin and cyclic-adenosine monophosphate during prolonged administration. *J Lab Clin Med* 1975; 86: 445–461.

17. Drew GM, Whiting SB. Evidence for two distinct types of postsynaptic alpha-adrenoceptor in vascular smooth muscle in vivo. *Br J Pharmacol* 1979; 67: 207–215.
18. Nielsen H *et al.* Age-dependent changes in alpha-adrenoceptor-mediated contractility of isolated human resistance arteries. *Am J Physiol* 1992; 263 (4 Pt 2): H1190–H1196.
19. Awe SO, Adeagbo AS. Vascular alpha1-adrenoceptors in isolated perfused rat kidney: influence of ageing. *Auton Autacoid Pharmacol* 2007; 27: 19–26.
20. Yaris E *et al.* The effects of paroxetine on rat isolated vas deferens. *Pharmacol Res* 2003; 48: 335–345.
21. Medina P *et al.* Effects of antidepressants in adrenergic neurotransmission of human vas deferens. *Urology* 2000; 55: 592–597.
22. Baldessarini RJ. Drug therapy of depression and anxiety disorders. In: Brunton LL *et al.*, ed. *Goodman and Gilman's the Pharmacological Basis of Therapeutics*, 11th edn. New York: Mc-Graw Hill, 2006: 429–459.
23. Tremaine LM *et al.* Metabolism and disposition of the 5-hydroxytryptamine uptake blocker sertraline in the rat and dog. *Drug Metab Dispos* 1989; 17: 542–550.
24. Kadioglu M *et al.* Paroxetine inhibited the relaxations induced by EFS in mice corpus cavernosum: is it a NOS inhibition? *Fundam Clin Pharmacol* 2010; 24: 55–61.
25. Seo KK *et al.* Comparison of peripheral inhibitory effects of clomipramine with selective serotonin re-uptake inhibitors on contraction of vas deferens: in vitro and in vivo studies. *J Urol* 2001; 165 (6 Pt 1): 2110–2114.
26. Minneman KP, Esbenshade TA. Alpha 1-adrenergic receptor subtypes. *Annu Rev Pharmacol Toxicol* 1994; 34: 117–133.
27. Berridge MJ. Inositol trisphosphate and calcium signalling mechanisms. *Biochim Biophys Acta* 2009; 1793: 933–940.
28. Hume JR *et al.* Caffeine inhibits InsP3 responses and capacitative calcium entry in canine pulmonary arterial smooth muscle cells. *Vascul Pharmacol* 2009; 50: 89–97.
29. Smani T *et al.* Complex regulation of store-operated Ca<sup>2+</sup> entry pathway by PKC-epsilon in vascular SMCs. *Am J Physiol Cell Physiol* 2008; 294: C1499–C1508.
30. Somlyo AP, Somlyo AV. Signal transduction and regulation in smooth muscle. *Nature* 1994; 372: 231–236.
31. Kalyoncu NI *et al.* Sertraline inhibits the contractile responses to noradrenaline, KCl and electrical field stimulation of rat isolated vas deferens. *J Auton Pharmacol* 1999; 19: 365–369.
32. Molin JC, Bendhack LM. Clonidine induces rat aorta relaxation by nitric oxide-dependent and -independent mechanisms. *Vascul Pharmacol* 2004; 42: 1–6.
33. Somlyo AP *et al.* Pharmacomechanical coupling: the role of calcium, G-proteins, kinases and phosphatases. *Rev Physiol Biochem Pharmacol* 1999; 134: 201–234.