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Blockade of calcium entry in smooth muscle cells by the antidepressant imipramine

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Abstract

The present study was designed to evaluate the effects of antidepressants on smooth muscle contractile activity. In rat aortic rings, the antidepressants imipramine, mianserin and sertraline provoked concentration-dependent inhibitions of the mechanical responses evoked by K^+ (30 mM) depolarization. These myorelaxant effects were not modified by the presence of glibenclamide or 80 mM K^+ in the bathing medium. Moreover, the vasodilator properties of imipramine were not affected by atropine, phentolamine and pyrilamine. Radioisotopic experiments indicated that imipramine failed to enhance ^{86}Rb outflow from prelabelled and perifused aortic rings whilst counteracting the increase in ^{45}Ca outflow provoked by a rise in the extracellular K^+ concentration. Simultaneous measurements of contractile activity and fura-2 fluorescence revealed that, in aortic rings, imipramine reduced the mechanical and fluorimetric response to K^+ challenge. In A7r5 smooth muscle cells, whole cell recordings further demonstrated that imipramine inhibited the inward Ca^{2+} current. Under different experimental conditions, the ionic and relaxation responses to the antidepressants were reminiscent of those mediated by the Ca^{2+} entry blocker verapamil. Lastly, it should be pointed out that imipramine exhibited a myorelaxant effect of similar amplitude on rat aorta and on rat distal colon. All together, these findings suggest that the myorelaxant properties of imipramine, and probably also setraline and mianserin, could result from their capacity to inhibit the voltage-sensitive Ca^{2+} channels.

Keywords: Antidepressants; Imipramine; Ca^{2+} entry blocker; Smooth muscle; Contractile activity

1. Introduction

Depression is a heterogeneous disorder exhibiting a quite high prevalence in the world's population [1]. Although the understanding of the physiology of mood disorders is still limited, chemical treatment of clinical depression is known to cause significant improvement in the great majority of patients [2].

Various classes of compounds with different chemical structures have been found to have antidepressant activity [1–3]. Tricyclic antidepressants, namely imipramine and imipramine-like compounds, have been widely used for the treatment of depression and can be referred as first-generation antidepressants. A second-generation and heterogeneous group includes, among other drugs, tetracyclic antidepressants such as mianserin. The compounds belonging to these pharmacological classes have been shown to block, mainly but not exclusively, the neuronal uptake of serotonin and norepinephrine [1,2]. Moreover, an original series of successful antidepressant agents has recently emerged and consists of drugs such as fluoxetine or sertraline which have been described as selective inhibitors of the reuptake of serotonin into nerve terminals. Thus, this series of compounds has been named ''selective serotonin reuptake inhibitors''. Besides these three groups of compounds, other drugs including atypical antidepressants and monoamine oxidase inhibitors are also effective in treating depression [1,2].

Most of the available antidepressants exhibit a limited therapeutic index and numerous side-effects have been reported. Autonomic, neurologic, cardio-vascular, gastro-intestinal and metabolic adverse effects are common [1–3]. Changes in brain monoaminergic neurotransmission as well as the capacity of antidepressants to block muscarinic, adrenergic or histaminergic receptors can

Abbreviations: $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; FOR, fractional outflow rate; K_{ATP}, ATP-sensitive K^+; TTX, tetrodotoxin

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contribute to the broad range of side-effects and/or toxicity [2,3]. Other mechanisms, including alterations of transmembrane movement of ions, can, however, also be involved [4–7].

Because some adverse-effects of antidepressants could be related to a direct action on smooth muscle, the present study was designed to evaluate the effect of imipramine, sertraline and mianserin on the mechanical activity of the rat aorta and the rat distal colon. In addition, different technical and pharmacological approaches have been combined to fully characterize the mechanism of action of imipramine on vascular smooth muscle cells.

2. Materials and methods

2.1. Measurements of isometric contractions in rat aorta and rat colon

The laboratory animal care was approved by the ethics committee of the Université Libre de Bruxelles.

Adult fed female Wistar (200–220 g, Charles River Laboratories) rats were exsanguinated. The thoracic aorta was cut into transverse rings (2–3 mm). Adhering fat, connective tissue and endothelium were removed. The distal part of the colon was also isolated, cut into two parts and adhering fat as well as connective tissue were removed. In both cases, the segments were suspended under 2 g load in an organ bath containing Krebs bicarbonate-buffered solution (20 ml; in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5). The solution maintained at 37 °C was continuously oxygenated with a mixture of 95% O_2 and 5% CO_2. After equilibration for 60 min (aortic rings) or 30 min (colon), isometric contractions were measured with a force-displacement transducer. In some experiments, contractile activity was induced by increasing the extracellular concentration of K^+ (30 or 80 mM KCl). When a plateau of tension was reached, drugs were added to the preparation cumulatively until maximal relaxation. Some experiments were repeated in the continuous presence of 1 or 10 μM glibenclamide.

For the measurement of mechanical activity in rat distal colon, tetrodotoxin (TTX, 0.5 μM) was added to the bathing media in order to block the neuronal activity of the preparation [8].

2.2. Measurements of ^{45}Ca and ^{86}Rb outflow from perifused rat aorta rings

Experiments were performed with thoracic aorta rings (≅2 mm length) isolated from adult fed female Wistar rats (200–220 g). The aorta rings were preincubated for 30 min in a bicarbonate buffered medium (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) containing 5 mM glucose and further incubated for 60 min in the same medium containing, in addition, either ^{45}Ca ion (0.02–0.04 mM; 100 μCi/ml) or ^{86}Rb (0.15–0.25 mM; 50 μCi/ml).

The method used to measure ^{45}Ca or ^{86}Rb (^{42}K substitute) outflow from rat perifused aortic rings has been described previously [9–11].

The outflow of ^{45}Ca or ^{86}Rb ion (cpm/min) was expressed as a fractional outflow rate (% of instantaneous aorta content/min, FOR).

2.3. Simultaneous measurements of rat aortic contractile activity and cytosolic Ca^{2+} concentration by front-surface fluorimetry

Aortic rings isolated from adult fed female Wistar rats (200–220 g) were inverted and rubbed to remove the endothelium. The rings were incubated for 3–4 h at room temperature in a physiological solution (in mM: NaCl, 122.9; $NaHCO_3$, 15; KCl, 5; $CaCl_2$, 1.25; $MgCl_2$, 1.2; glucose, 11) containing 5 μM fura-2 acetoxymethyl ester (fura-2 AM, Calbiochem) and 0.05% Cremophor EL (Sigma-Aldrich), and further incubated for 30 min in a fura-2 and Cremophor EL-free solution [12]. Rings were mounted between two hooks at a tension of 20 mN in a 3 ml organ bath filled with the physiological solution, at 37 °C, gassed with 95% O_2 and 5% CO_2. The bath was part of a fluorimeter (CAF 110, JASCO) used to measure the fluorescent signal. After stabilization of the tension during 30 min, the 5 mM K^+ solution in the organ bath was replaced with a 80 mM K^+ solution for 10 min. Following this depolarizing pulse, 10 μM verapamil, 10 μM imipramine or the required amount of solvent (control experiment) were added to the physiological medium (K^+ 5 mM). Twenty minutes later, a second 80 mM K^+ pulse was elicited for 10 min in the continuous presence of either verapamil or imipramine. The muscle tone was measured by an isometric force transducer. Moreover, during the measurement of contractile activity, the luminal face of the aortic ring was alternatively illuminated (128 Hz) with two excitation wavelengths (340 ± 10 and 380 ± 10 nm) obtained from a xenon high pressure lamp (75 W) coupled to two monochromators. The emitted light from the muscle was collected by a photomultiplier through a 500 ± 12 nm filter. The fluorescence signals due to excitation at 340 nm (F_{340}) and at 380 nm (F_{380}) as well as the ratio F_{340}/F_{380} (representative of the cytosolic calcium concentration ($[Ca^{2+}]_i$)) were simultaneously measured with contractile tension and recorded using data acquisition hardware (MacLab) and data recording software (Chart v3.3, AD Instruments Pty. Ltd.). The autofluorescence was measured at 340 and 380 nm by quenching the fura-2 fluorescence with $MnCl_2$ (10 mM) and was subtracted from the experimental values.

Each experiment was repeated on at least four to six different rat aortic rings.

2.4. Ca^{2+} channel current recording from A7r5 cells

Voltage-clamp experiments were performed at room temperature in the whole-cell configuration of the patch-clamp technique [13]. The A7r5 aortic smooth muscle cells were obtained from European Collection of Cell Culture (ECACC). The cells were cultured at 37 °C in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 2 mM glutamine and 10% fetal bovine serum (Gibco).

Pipettes (2–5 MΩ) were pulled and polished using a DMZ-universal puller (Zeitz Instrument Vertriebs GmbH) and connected to the head stage of a patch-clamp amplifier (List EPC-7). Programmed voltage-clamp sequences and data acquisition were performed by specific software (pClamp software, Axon Instruments) through an A/D-D/A conversion board (Labmaster, Scientific solutions). Ba^{2+} current was elicited by depolarizing steps, 200–500 ms in duration, every 30 s. The holding potential was −70 mV. The pipette was filled with a K^+-free solution containing (in mM): NaCl 120, $MgCl_2$ 4, glucose 10, EGTA 3, Na_2ATP 5, HEPES 10, pH adjusted to 7.2 with NaOH. The bath was continuously perfused with a physiological salt solution containing (in mM): NaCl 120, $MgCl_2$ 1.2, $BaCl_2$ 10, glucose 10, HEPES 10, pH adjusted to 7.4 with NaOH. Substitution of K^+ by Na^+ in both pipette and external solutions set the reversal potential of Na^+ and Cl^- close to 0 mV. For this reason, the test voltage of current measurement was set at 0 mV [14]. Experiments were repeated on 11 different cells.

2.5. Drugs

According to the experiments, the media were enriched with imipramine (Sigma-Aldrich), sertraline (Pfizer), mianserin (Organon), atropine (Sigma-Aldrich), phentolamine (Sigma-Aldrich), pyrilamine (Sigma-Aldrich), serotonin (5-hydroxytryptamine, ICN Biomedicals), glibenclamide (ICN Biomedicals), phenylephrine (Acros Organics) or tetrodotoxin (TTX, Acros Organics).

Glibenclamide and sertraline were dissolved in dimethylsulphoxide which was added to both control and test media. At the final concentrations used (<0.5%), dimethylsulphoxide failed to affect smooth muscle contractility [9,10]. Serotonin and TTX were dissolved in a 150 mM NaCl solution. When high concentrations of extracellular K^+ (80 mM) were used, the concentration of extracellular NaCl was lowered to keep osmolarity constant.

2.6. Calculations

Results are expressed as the mean ± S.E. Contractile responses were expressed as the percentage of the KCl response. IC_{50} values (drug concentrations inducing half-maximum inhibition of the plateau phase induced by KCl) were assessed from concentration–response curves using Datanalyst software (EMKA Technologies). Peak ^{45}Ca outflow was estimated from the difference in ^{45}Ca outflow between the highest value recorded during stimulation and the mean basal value found within the same experiment between the 40th and 44th min of perifusion. For the simultaneous measurement of myogenic activity and $[Ca^{2+}]_i$, the concomitant increase in muscle tension and $[Ca^{2+}]_i$ was estimated in each individual experiment from the integrated value measured during the 6 first min of stimulation after correction for basal value (1–2 min before stimulation). The contraction and increase in $[Ca^{2+}]_i$ elicited by the first exposure to high K^+ was considered as the control value (100%).

The statistical significance of the differences between mean data was assessed by use of paired or unpaired Student's *t*-test.

3. Results

3.1. Effects of imipramine, sertraline and mianserin on the contractile activity of rat aortic rings

In rat aortic rings exposed to 30 mM K^+, the cumulative application of imipramine, sertraline or mianserin (0.1 μM–0.3 mM) induced concentration-dependent relaxations (Fig. 1). According to the IC_{50} values (Table 1), imipramine and sertraline were approximately four to five times more potent than diazoxide (a reference ATP-sensitive K^+ (K_{ATP}) channel opener [15,16]) at reducing the vascular tone ($P < 0.001$). By comparison, mianserin was roughly as potent as the K_{ATP} channel opener ($P > 0.05$) (Table 1). The three antidepressants, as well as diazoxide, were however less active than the Ca^{2+} entry blocker verapamil ($P < 0.01$) (Table 1) [17,18].

Further experiments were conducted in order to characterise the myorelaxant effect of imipramine, sertraline and mianserin. First, in aortic rings exposed to 30 mM K^+,

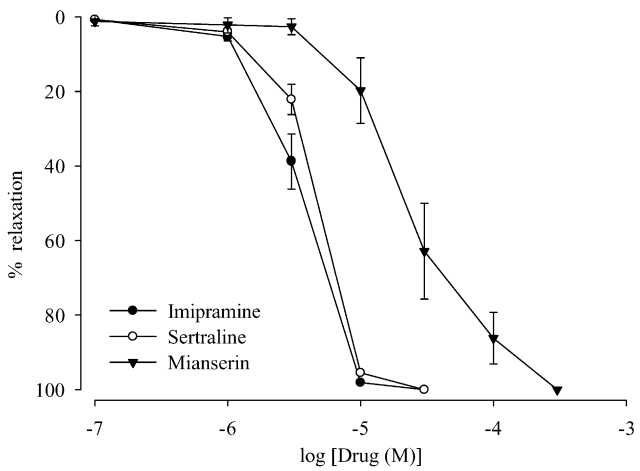

Fig. 1. Dose–action curves of imipramine, sertraline and mianserin on KCl (30 mM)-induced contraction of rat aortic rings. Mean values ± S.E. refer to four to six individual experiments.

Table 1
IC_{50} values in response to imipramine, sertraline, mianserin, diazoxide and verapamil in K^+-depolarized rat aortic rings

Compound	30 mM KCl	30 mM KCl + 1 μM GLIB	30 mM KCl + 10 μM GLIB	80 mM KCl
Imipramine	3.8 ± 0.4 (6)	4.3 ± 0.1 (4)	4.1 ± 0.5 (4)	2.5 ± 0.3 (4)
Sertraline	4.7 ± 0.2 (6)	05.4 ± 0.2 (4)*	5.6 ± 0.5 (4)	4.8 ± 0.3 (4)
Mianserin	16.7 ± 2.7 (6)	21.7 ± 2.4 (6)	23.0 ± 2.6 (6)	17.8 ± 1.4 (6)
Diazoxide[a]	19.5 ± 2.7 (5)	85.8 ± 22.2 (5)*	163.4 ± 41.2 (5)**	>300 (5)***
Verapamil[a]	0.061 ± 0.024 (4)	0.074 ± 0.021 (4)	0.067 ± 0.016 (4)	0.053 ± 0.015 (4)

IC_{50} is the drug concentration (μM) eliciting 50% relaxation of the 30 or 80 mM KCl-induced contraction of rat aortic rings. Number in parentheses refer to the number of experiments performed in each group. GLIB: glibenclamide. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.
[a] From Ouedraogo et al. [18].

the relaxation response to imipramine was not modified by the presence of atropine (0.5 μM), phentolamine (0.5 μM) and pyrilamine (0.5 μM) in the bathing medium (data not shown). When concentration–response curves were performed in the simultaneous presence of atropine, phentolamine and pyrilamine, the IC_{50} value for imipramine averaged 3.4 ± 0.6 μM ($n = 3$; $P > 0.5$). Secondly, we tested the effects of imipramine, sertraline and mianserin on rat aortic rings incubated in the presence of either 1 or 10 μM of the hypoglycemic sulfonylurea glibenclamide, a K_{ATP}-channel blocker [19,20] (data not shown). Table 1 clearly indicates that the vasorelaxant effects of the three drugs were not affected by the presence of glibenclamide in the incubation medium. By contrast, incubation of the preparations with glibenclamide provoked a dose-dependent rightward shift of the diazoxide, but not the verapamil, concentration–response curves (Table 1). Incidentally, the presence of glibenclamide (1, 10 μM) in the medium did not affect the baseline tension or the contractile responses to KCl (30 mM) (data not shown).

In the next series of experiments, contractile activity was elicited by high concentrations of extracellular K^+ (80 mM) (data not shown). Under such experimental conditions, the vasorelaxant properties of imipramine, sertraline and mianserin remained unchanged (Table 1). Moreover, in rat aortic rings precontracted with 80 mM K^+, the vasorelaxant effects of verapamil persisted whilst those of diazoxide were almost completely abolished (Table 1).

Finally, imipramine, sertraline and mianserin (0.1 μM–0.3 mM) did not affect the mechanical activity of aortic rings bathed in a medium containing 5 mM extracellular K^+ (data not shown).

3.2. Effects of serotonin and phenylephrine on the contractile activity of rat aortic rings

Figs. 2 and 3 (upper panels) illustrate the effects of increasing concentrations of serotonin and phenylephrine on the muscle tension of aortic rings exposed to 30 mM KCl. Neither the cumulative application of serotonin (10 nM–100 μM) nor that of phenylephrine (10 nM–3 μM) induced a relaxation. As opposed to the antidepressants tested under identical experimental conditions, both serotonin and phenylephrine provoked a small and sustained increase in muscle tone. Likewise, in aortic rings exposed to 5 mM extracellular K^+, the cumulative application of serotonin (10 nM–30 μM) or phenylephrine (10 nM–10 μM) induced concentration-dependent contractions (Figs. 2 and 3, lower panels). At 10 and 30 μM, serotonin elicited phasic contractions (Fig. 2, lower panel).

3.3. Effects of imipramine on ^{86}Rb outflow from rat aortic rings

In the presence of 30 mM K^+ in the basal medium, the addition of 10 μM imipramine did not affect the ^{86}Rb outflow from prelabelled and perifused rat aortic rings (data not shown).

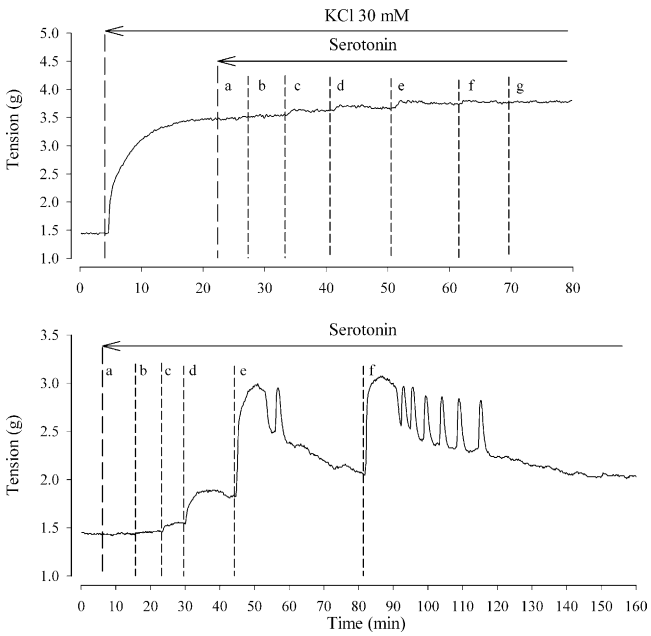

Fig. 2. Effect of increasing concentrations of serotonin [(a) 10 nM, (b) 100 nM, (c) 1 μM, (d) 3 μM, (e) 10 μM, (f) 30 μM, and (g) 100 μM] on the contractile activity of rat aortic rings exposed to a physiological medium containing either 30 mM (upper panel) or 5 mM KCl (lower panel). Each graph is a representative experiment conducted on a single rat aortic ring.

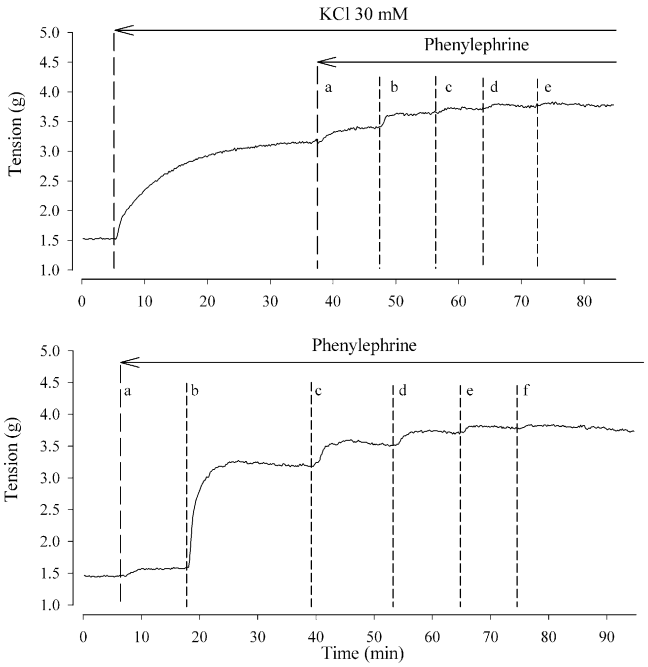

Fig. 3. Effect of increasing concentrations of phenylephrine [(a) 10 nM, (b) 100 nM, (c) 300 nM, (d) 1 μM, (e) 3 μM, and (f) 10 μM] on the contractile activity of rat aortic rings exposed to a physiological medium containing either 30 mM (upper panel) or 5 mM KCl (lower panel). Each graph is a representative experiment conducted on a single rat aortic ring.

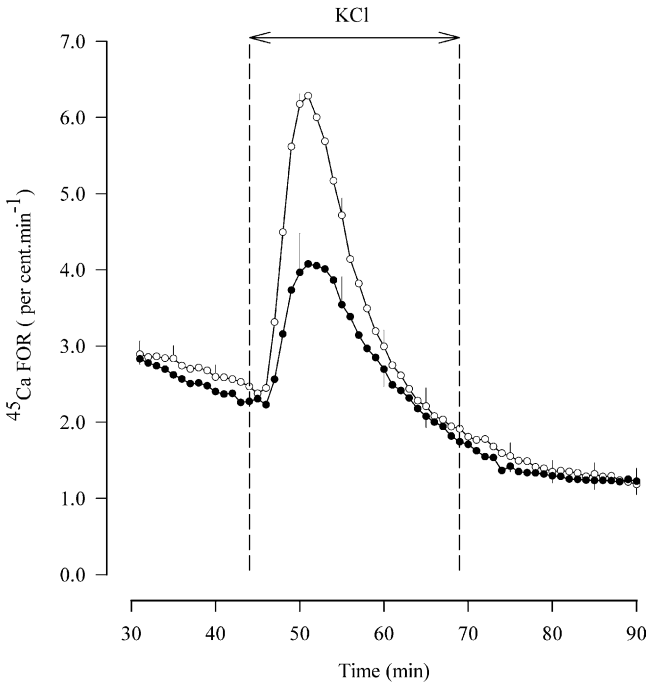

Fig. 4. Effects of KCl 30 mM on ^{45}Ca outflow from rat aortic rings perifused throughout in the absence (○) or presence of imipramine (●; 10 μM). Mean values ± S.E. refer to seven individual experiments.

3.4. Effects of imipramine and verapamil on KCl-induced changes in ^{45}Ca outflow from rat aortic rings

A rise in the extracellular concentration of K^+ from 5 to 30 mM provoked a rapid and marked increase in ^{45}Ca outflow from prelabelled and perifused rat aortic rings (Fig. 4).

When imipramine (10 μM) was present in the perifusate, the stimulatory effect of 30 mM K^+ was reduced (Fig. 4). The peak ^{45}Ca outflow recorded after K^+ stimulation averaged 3.9 ± 0.3% per min ($n = 7$) in the absence and 1.9 ± 0.4% per min ($n = 7$) in the presence of 10 μM imipramine ($P < 0.01$).

In another series of experiments, we characterised the effects of imipramine on the ^{45}Ca response to 80 mM K^+ (Fig. 5, left panel). As previously described [9], raising the extracellular K^+ concentration from 5 to 80 mM again provoked an immediate and pronounced increase in ^{45}Ca FOR. When the same experiment was repeated in the presence of 10 μM imipramine in the perifusing medium, the enhancing effect of 80 mM K^+ was decreased. Thus, the peak ^{45}Ca outflow averaged 4.8 ± 0.2% per min ($n = 6$) in the absence and 2.2 ± 0.2% per min ($n = 6$) in the presence of 10 μM imipramine ($P < 0.01$).

For comparison, we also characterised the effects of the Ca^{2+} channel blocker verapamil [17] on the ^{45}Ca response to high extracellular K^+ (Fig. 5, right panel). The presence of verapamil (10 μM) in the perifusate completely abolished the rise in ^{45}Ca FOR evoked by the increment in extracellular K^+.

3.5. Simultaneous measurements of the effects of imipramine and verapamil on the contractile activity and the cytosolic Ca^{2+} concentration of rat aortic rings

Iterative rises in the extracellular concentration of K^+ from 5 to 80 mM, separated by a washing period lasting at least 10 min, provoked similar rapid and marked increases in muscle tone and cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) (Fig. 6). In control experiments (Fig. 6, left panels), the increments in muscle tension and $[Ca^{2+}]_i$ provoked by the second stepwise increase in extracellular K^+ concentration averaged 108.6 ± 11.6% ($n = 3$) ($P > 0.05$) and 103.7 ± 13.5% ($n = 3$) ($P > 0.05$) of the initial K^+ pulse, respectively.

In aortic rings exposed to verapamil or imipramine, the mechanical and fluorimetric responses to 80 mM K^+ were markedly reduced (Fig. 6, right panels, and Fig. 7). In the continuous presence of 10 μM verapamil in the basal medium (Fig. 6, right panels), the magnitude of the increases in contractile activity and $[Ca^{2+}]_i$ provoked by the second K^+ challenge represented 3.4 ± 1.2% ($n = 3$) ($P < 0.001$) and 9.8 ± 1.4% ($n = 3$) ($P < 0.001$) of the control value, respectively. Likewise, when the bathing medium contained 10 μM imipramine (Fig. 7), the magnitude of the K^+-induced increase in contractile activity and $[Ca^{2+}]_i$ amounted to 8.6 ± 1.0% ($n = 3$) ($P < 0.001$) and 37.6 ± 6.4% ($n = 3$) ($P < 0.001$) of the control value.

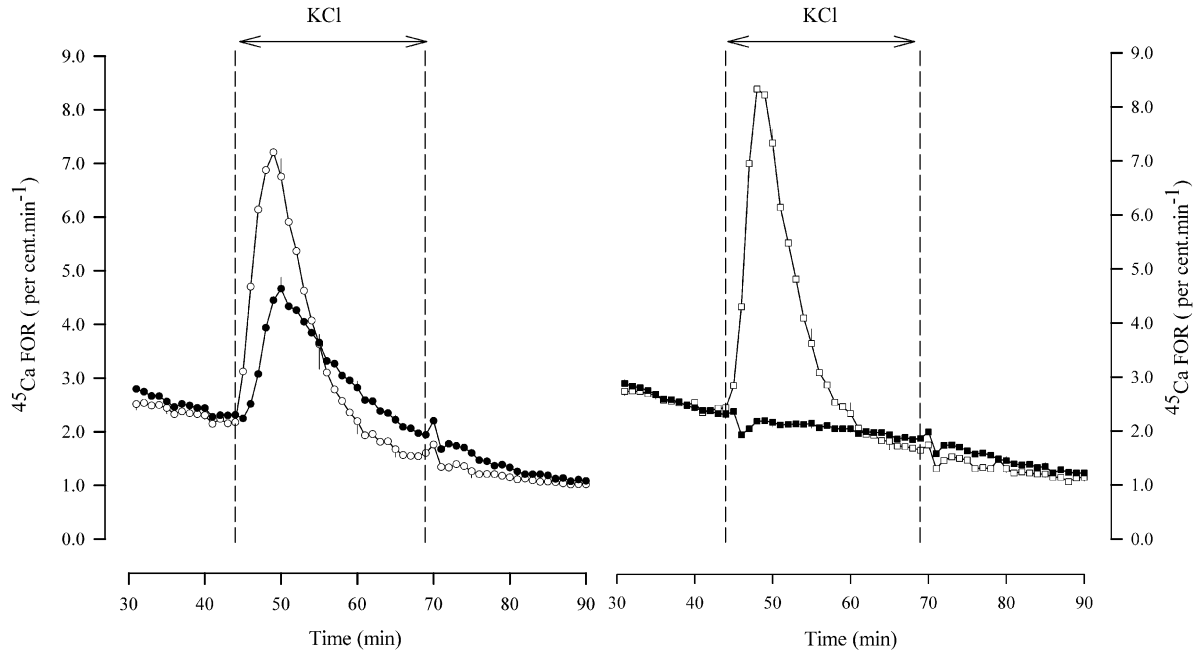

Fig. 5. Effects of KCl 80 mM on ^{45}Ca outflow from rat aortic rings perifused throughout in the absence (○, □) or presence of imipramine (●; 10 μM; left panel) or verapamil (■, 10 μM; right panel). Mean values ± S.E. refer to six (left panel) and four to seven individual experiments (right panel).

3.6. Effect of imipramine on inward Ca^{2+} current in A7r5 cells

In A7r5 aortic smooth muscle cells bathed in K^+-free solution and with Ba^{2+} as charge carrier, the predominant voltage-dependent inward current is a dihydropyridine-sensitive L-type calcium current [21]. After establishment of the whole cell configuration, the current was elicited by brief depolarising test pulses from a holding potential of −70 to 0 mV. As illustrated in Fig. 8 (left panel), the application of imipramine (10 μM) in the bathing solution produced a marked and reversible decrease in the inward

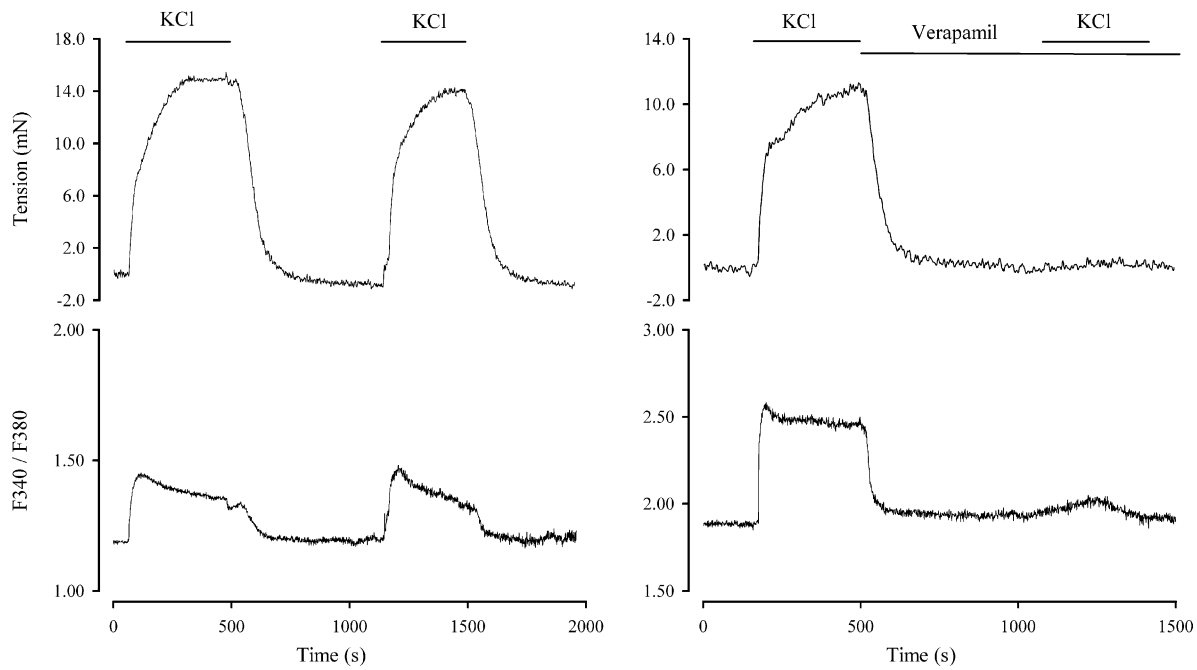

Fig. 6. Effects of two successive increases in the extracellular concentration of K^+ from 5 to 80 mM on the contractile activity (upper panels) and F_{340}/F_{380} ratio (lower panels) of fura-2 loaded rat aortic rings. The second rise in extracellular K^+ occured in the absence (left panels) or presence of verapamil in the basal medium (right panels; 10 μM). Graphs are representative experiments conducted on a single aortic ring. Tension and fluorescences are collected in parallel on the same aortic ring.

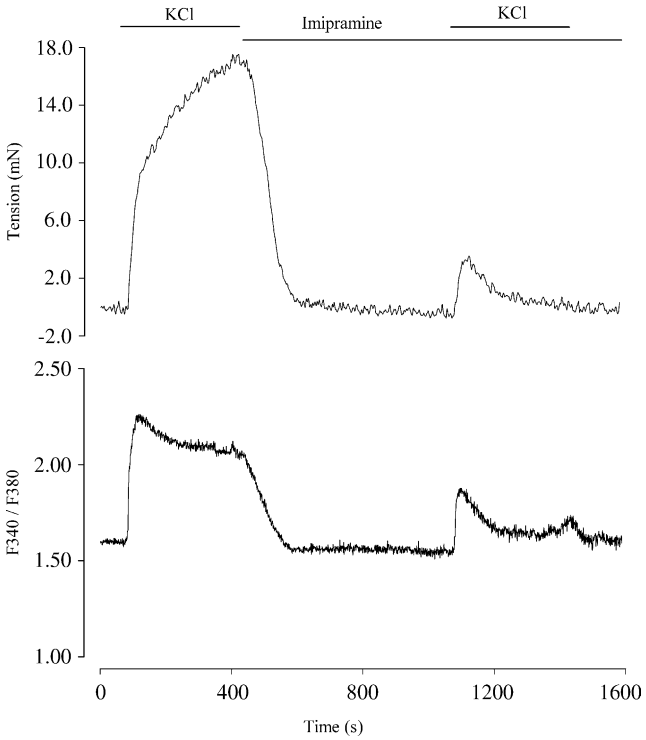

Fig. 7. Effects of two successive increases in the extracellular concentration of K^+ from 5 to 80 mM on the contractile activity (upper panel) and F_{340}/F_{380} ratio (lower panel) of a fura-2 loaded rat aortic ring. The second rise in extracellular K^+ occured in the presence of imipramine (10 μM) in the basal medium. The graph is a representative experiment conducted on a single aortic ring. Tension and fluorescences are collected in parallel on the same aortic ring.

Ca^{2+} current. At steady state, imipramine inhibited the current by 75 ± 5%, from −2.3 ± 0.3 pA/pF to −0.6 ± 0.2 pA/pF ($n = 11$ cells). Fig. 8 (right panel) also shows the current–voltage relation obtained by applying increasing depolarizing pulses from a holding potential of −70 mV in the absence and in the presence of imipramine (10 μM).

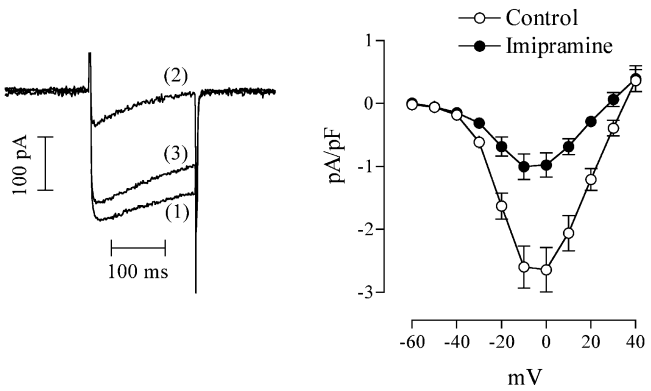

Fig. 8. (Left panel) Typical recording of current traces observed during a depolarizing pulse to 0 mV in a A7r5 cell. Current before (1), in the continuous presence (2) and 15 min after the removal (3) of imipramine (10 μM) from the perifusion medium. (Right panel) Effect of imipramine (10 μM) on the current–voltage relation for peak inward current before and after application of the drug. Current was normalised for cell capacitance. Mean values ± S.E. refer to five individual experiments.

The current–voltage relation was markedly depressed in the presence of imipramine in the external solution.

3.7. Effects of imipramine on the contractile activity of rat distal colon

Fig. 9 (upper panel) illustrates the effect of increasing concentrations of imipramine on the muscle tension of rat distal colon continuously exposed to 5 mM K^+ and 0.5 μM tetrodotoxin (TTX). As previously described [22], the spontaneous tone of the distal colon was enhanced by the presence of TTX in the bathing medium. The amplitude as well as the frequency of the phasic contractions elicited by the addition of TTX did not, however, exhibit a regular pattern [22]. Under such experimental conditions, 30 μM imipramine inhibited the spontaneous myogenic activity. The withdrawal of imipramine from the medium slowly induced the reappearance of phasic contractions (data not shown).

In the next series of experiments, the distal colon was exposed to 30 mM K^+ in the continuous presence of 0.5 μM TTX (Fig. 9). Raising the extracellular K^+ concentration elicited an initial phasic contraction followed by a sustained plateau (Fig. 9, lower panel). The cumulative addition of imipramine (1–10 μM) during the tonic component of the contractile response to KCl provoked concentration-dependent relaxations (Fig. 9, lower panel). The myorelaxant effect of imipramine was not modified by atropine (0.5 μM), phentolamine (0.5 μM) or pyrilamine (0.5 μM) (data not shown). The IC_{50} value developed in response to imipramine amounted to 1.5 ± 0.3 μM ($n = 4$) in the absence and 2.0 ± 0.1 μM ($n = 6$) in the simultaneous presence of the three receptor antagonists in the bathing medium ($P > 0.05$).

Lastly, we characterised the effects of serotonin (10 nM–10 μM) and phenylephrine (1 nM–1 μM) on the contractile response to KCl. In rat distal colon exposed to 30 mM K^+ and 0.5 μM TTX, both amines failed to affect the muscle tone (data not shown).

4. Discussion

Our data clearly reveal the ability of imipramine, sertraline and mianserin to provoke concentration-dependent inhibitions of the mechanical responses evoked by K^+(30 mM) depolarization in rat aortic rings. The vasorelaxant effects of imipramine and sertraline were more marked than that of diazoxide, whilst mianserin was as potent as the reference K_{ATP} channel opener [9,15,16]. By contrast, verapamil, a prototype phenylalkylamine Ca^{2+} entry blocker [17], was more potent than the three antidepressants at reducing the vascular tone.

The vasodilator properties of imipramine, sertraline and mianserin cannot be ascribed to an interference at a serotoninergic, an α adrenergic, an histaminergic or a

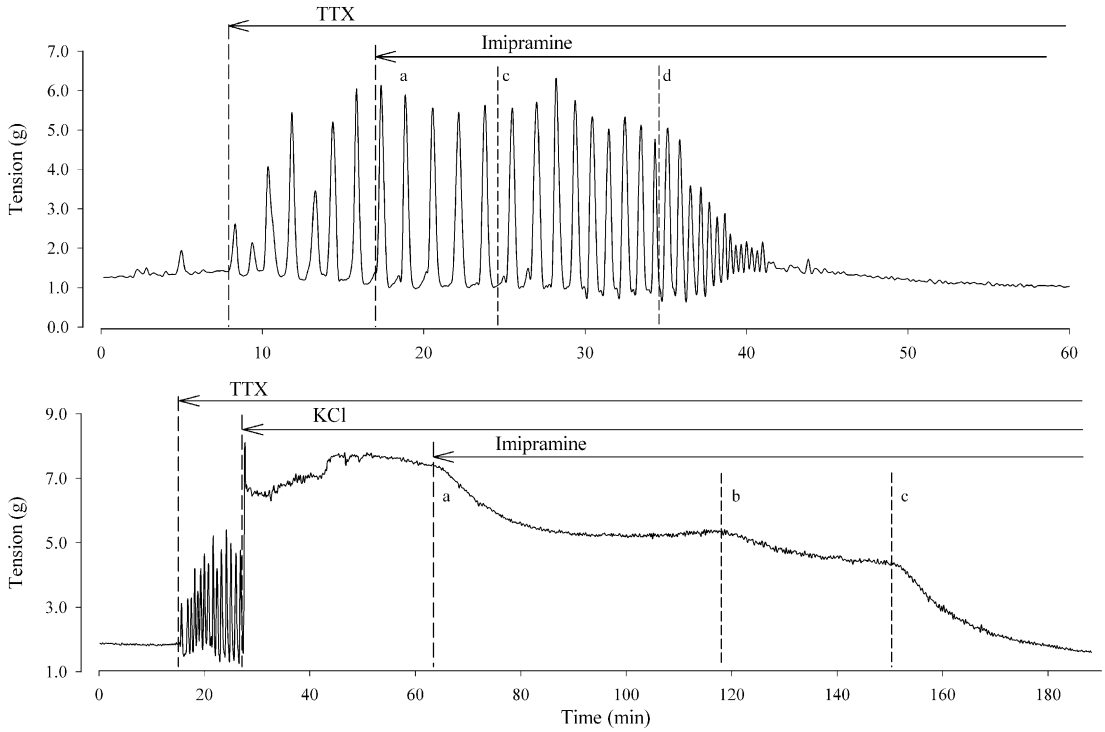

Fig. 9. Effects of increasing concentrations of imipramine [(a) 1 μM, (b) 3 μM, (c) 10 μM, and (d) 30 μM] on the contractile activity of rat distal colon exposed to a physiological medium containing either 5 mM (upper panel) or 30 mM KCl (lower panel); in the presence of TTX (0.5 μM) throughout. Each graph is a representative experiment conducted on a single part of rat distal colon.

muscarinic binding site. Indeed, whether the bathing medium contained 5 or 30 mM extracellular K^+, both serotonin and phenylephrine provoked an increase in aortic muscle tone [23,24]. Moreover, in rat aortic rings exposed to 30 mM K^+, the response to imipramine was not modified by the presence of atropine (muscarinic antagonist), phentolamine (α adrenergic antagonist) and pyrilamine (histaminergic antagonist) in the bathing medium [23].

The present data rather suggest that the mechanical responses to the antidepressants could be related to their capacity to affect transmembrane ionic movements. In agreement with such a view, the antidepressants-induced vascular relaxations were detected on K^+ depolarized rat aortic rings and the myorelaxant effects of imipramine, sertraline or mianserin were unchanged whether the bathing medium contained 30 or 80 mM extracellular K^+. An increase in extracellular concentration of K^+, by changing the Nernst equilibrium potential, is known to depolarize the smooth muscle cells which in turn activates the voltage-sensitive Ca^{2+} channels and allows Ca^{2+} inflow [22,23]. Moreover, under the different experimental conditions, the relaxation responses to the three antidepressant agents were reminiscent of those mediated by verapamil, a Ca^{2+} entry blocker [17,18]. Indeed, in vascular smooth muscle cells like in other cell types, voltage-sensitive Ca^{2+} channel blockers are capable of inhibiting the physiological responses evoked by a "low" but also by a "high" extracellular K^+ concentration [10,16,25].

Additional experiments conducted with imipramine confirmed that, in smooth muscle cells, antidepressants could behave as Ca^{2+} entry blockers.

First, the antidepressant imipramine reduced the KCl-induced increase in ^{45}Ca outflow from prelabeled and perifused rat aortic rings. This cationic response to K^+ is known to reflect a process of ^{40}Ca–^{45}Ca exchange into which influent ^{40}Ca displaces ^{45}Ca from intracellular binding sites [9,26]. The inhibitory effect of imipramine persisted in the presence of a high extracellular K^+ concentration and was reproduced by the Ca^{2+} entry blocker verapamil. Such data indirectly suggest that the effects of imipramine on the KCl-induced changes in ^{45}Ca FOR result from an inhibition of $^{40}Ca^{2+}$ entry into smooth muscle cells.

Second, patch-clamp recordings conducted on A7r5 cells indicated that imipramine markedly reduced an inward Ca^{2+} current.

Finally, further evidence supporting an inhibitory effect of imipramine on voltage-sensitive Ca^{2+} channels came from the simultaneous measurement of contractile activity and cytosolic Ca^{2+} concentration. In aortic rings exposed to iterative increases in external K^+, both imipramine and verapamil reduced the mechanical and fluorimetric response to K^+ challenge. The latter experiments also revealed the relationship between the modifications in cytosolic Ca^{2+} activity and muscle tension.

Altogether, these findings indicate that the vasodilator properties of imipramine, and probably also sertraline and

mianserin, can be interpreted as the result of a decrease in membrane Ca^{2+} permeability.

A decrease in Ca^{2+} entry can be viewed, under physiological conditions, as a direct effect of drugs at the level of Ca^{2+} channels or as a result of K^+ channel activation leading to subsequent decrease in Ca^{2+} channel activity. Using ^{86}Rb outflow as a measure of K^+ channel activity [10,11,27], we failed to detect any stimulatory effect of imipramine on the ^{86}Rb fractional outflow rate from prelabeled aortic rings. Moreover, prior exposure of aortic rings to the K^+ channel blocker glibenclamide did not affect the relaxant potency of imipramine, sertraline or mianserin. By contrast, the vasorelaxant properties of the K_{ATP} channel opener diazoxide were counteracted by glibenclamide. Such findings, as well as the close similarities between the effects of verapamil and imipramine under the experimental conditions so far examined, support the idea that imipramine, and probably also sertraline and mianserin, interacts at the level of voltage-gated Ca^{2+} channels. Voltage clamp experiments on A7r5 smooth muscle cells further substantiated this proposal. Incidentally, the present data extend previous observations suggesting an inhibitory effect of some antidepressants on a voltage-sensitive modality of Ca^{2+} entry [4,6,7,28,29]. Such drugs have also been reported to affect Na^+ [5,30] and/or K^+ channels [4,30–32].

The myorelaxant potency of imipramine was not restricted to aortic tissue. Indeed, and whether the bathing medium contained 5 or 30 mM extracellular K^+, the addition of imipramine inhibited the contractile activity of the isolated rat distal colon. This effect was maintained in the presence of tetrodotoxin, atropine, phentolamine and pyrilamine. Moreover, in muscle preparations exposed to tetrodotoxin and 30 mM K^+, serotonin and phenylephrine did not affect the muscle tone. Thus, such findings again rule out a prejunctional effect of imipramine and a role of serotoninergic, α adrenergic, histaminergic or muscarinic receptors.

Lastly, it should also be stressed that, under close to identical experimental conditions, imipramine exhibited a myorelaxant effect of similar amplitude on vascular and gastro-intestinal tissues.

In conclusion, the present data indicate that antidepressants with different chemical structures exhibit myorelaxant properties in smooth muscle preparations. Moreover, radioisotopic, electrophysiologic, fluorimetric and pharmacological observations further suggest that imipramine behaves as a Ca^{2+} entry blocker. The inhibition of voltage-sensitive Ca^{2+} channels reduces Ca^{2+} entry, causes a decrease in cytosolic Ca^{2+} concentration and, ultimately, impairs the muscle tone.

Even though the recommended therapeutic plasma concentrations are lower than those used in the present "in vitro" studies, it should be emphasized that antidepressants are known to accumulate in cells and to exhibit large apparent volumes of distribution [33,34]. Thus, it is tempting to speculate that some peripheral adverse-effects of antidepressants could be related, at least in part, to their capacity to affect Ca^{2+} channel activity with subsequent changes in cellular excitability.

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References

[1] Hollister LE. Antidepressant agents. In: Katzung BG, editor. Basic and clinical pharmacology. Connecticut: Appleton and Lange; 1995. p. 448–59.
[2] Frazer A. Pharmacology of antidepressants. J Clin Psychopharmacol 1997;17:2S–18S.
[3] Baldessarini RJ. Current status of antidepressants: clinical pharmacology and therapy. J Clin Psychiatry 1989;50:117–26.
[4] Isenberg G, Tamargo J. Effect of imipramine on calcium and potassium currents in isolated bovine ventricular myocytes. Eur J Pharmacol 1985;108:121–31.
[5] Pancrazio JJ, Kamatchi GL, Roscoe AK, Lynch III C. Inhibition of neuronal Na^+ channels by antidepressant drugs. J Pharmacol Exp Ther 1998;284:208–14.
[6] Vila JM, Medina P, Segarra G, Lluch P, Pallardo F, Flor B, et al. Relaxant effects of antidepressants on human isolated mesenteric arteries. Br J Clin Pharmacol 1999;48:223–9.
[7] Watts JA, Yates KM, Badar SK, Marcengill MB, Kline JA. Mechanisms of Ca^{2+} antagonism in imipramine-induced toxicity of isolated adult rat cardiomyocytes. Toxicol Appl Pharmacol 1998;153:95–101.
[8] Gershon MD. Effects of tetrodotoxin on innervated smooth muscle preparations. Br J Pharmacol 1967;29:259–79.
[9] Antoine M-H, Berkenboom G, Fang ZY, Fontaine J, Herchuelz A, Lebrun P. Mechanical and ionic response of rat aorta to diazoxide. Eur J Pharmacol 1992;216:299–306.
[10] Becker B, Antoine M-H, Nguyen Q-A, Rigo B, Cosgrove KE, Barnes PD, et al. Synthesis and characterization of a quinolinonic compound activating ATP-sensitive K^+ channels in endocrine and smooth muscle tissues. Br J Pharmacol 2001;134:375–85.
[11] Lebrun P, Fang ZY, Antoine M-H, Herchuelz A, Hermann M, Berkenboom G, Fontaine J. Hypoglycemic sulfonylureas antagonize the effects of cromakalim and pinacidil on ^{86}Rb fluxes and contractile activity in the rat aorta. Pharmacology 1990;41:36–48.
[12] Ghisdal P, Gomez J-P, Morel N. Action of a NO donor on the excitation-contraction pathway activated by noradrenaline in rat superior mesenteric artery. J Physiol 2000;522:83–96.
[13] Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 1981;391:85–100.
[14] Morel N, Buryi V, Feron O, Gomez JP, Christen MO, Godfraind T. The action of calcium channel blockers on recombinant L-type calcium channel α_1 subunits. Br J Pharmacol 1998;125:1005–12.
[15] Cook NS, Quast U. Potassium channel pharmacology. In: Cook NS, editor. Potassium channels, structure, classification, function and

therapeutic potential. Chichester: Ellis Horwood; 1990. p. 181–255.
[16] Edwards G, Weston AH. The pharmacology of ATP-sensitive potassium channels. Annu Rev Pharmacol Toxicol 1993;33:597–637.
[17] Godfraind T, Miller R, Wibo M. Calcium antagonism and calcium entry blockade. Pharmacol Rev 1986;38:321–416.
[18] Ouedraogo R, Fontaine J, Antoine M-H, Pirotte B, Lebrun P. Effects of 3-alkylamino-7-chloro-4*H*-pyrido[2,3-*e*]-1,2,4-thiadiazine 1,1-dioxides on smooth muscle contraction. Pharm Pharmacol Commun 2000;6:97–100.
[19] Malaisse WJ, Lebrun P. Mechanisms of sulfonylurea-induced insulin release. Diabetes Care 1990;13:9–17.
[20] Quayle JM, Nelson MT, Standen NB. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol Rev 1997; 77:1165–232.
[21] Buryi V, Morel N, Salomone S, Kerger S, Godfraind T. Evidence for a direct interaction of thapsigargin with voltage-dependent Ca^{2+} channels. Naunyn-Schmiedeberg's Arch Pharmacol 1995;351:40–5.
[22] Fontaine J, Lebrun P. Pharmacological analysis of the effects of BAY K 8644 and organic calcium antagonists on the mouse isolated distal colon. Br J Pharmacol 1988;94:1198–206.
[23] Bolton TB. Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 1979;59:606–718.
[24] Ratz PH, Flaim SF. Acetylcholine- and 5-hydroxytryptamine-stimulated contraction and calcium uptake in bovine coronary arteries: evidence for two populations of receptor-operated calcium channels. J Pharmacol Exp Ther 1985;234:641–7.
[25] Magnon M, Calderone V, Floch A, Cavero I. Influence of depolarization on vasorelaxant potency and efficacy of Ca^{2+} entry blockers, K^+ channel openers, nitrate derivatives, salbutamol and papaverine in rat aortic rings. Naunyn-Schmiedeberg's Arch Pharmacol 1998;358: 452–63.
[26] Godfraind T. Actions of nifedipine on calcium fluxes and contraction in isolated rat arteries. J Pharmacol Exp Ther 1983;224:443–50.
[27] Quast U, Baumlin Y. Comparison of the effluxes of $^{42}K^+$ and $^{86}Rb^+$ elicited by cromakalim (BRL 34915) in tonic and phasic vascular tissue. Naunyn-Schmiedeberg's Arch Pharmacol 1988;338:319–26.
[28] Kalyoncu NI, Ozyavuz R, Karaoglu S. Sertraline inhibits the contractile responses to noradrenaline. J Autonom Pharmacol 1999;19:365–9.
[29] Ungvari Z, Pacher P, Koller A. Serotonin reuptake inhibitor fluoxetine decreases arteriolar myogenic tone by reducing smooth muscle $[Ca^{2+}]_i$. J Cardiovasc Pharmacol 2000;35:849–54.
[30] Nicholson GM, Blanche T, Mansfield K, Tran Y. Differential blockade of neuronal voltage-gated Na^+ and K^+ channels by antidepressant drugs. Eur J Pharmacol 2002;452:35–48.
[31] Casis O, Gallego M, Sanchez-Chapula JA. Imipramine, mianserine and maprotiline block delayed rectifier potassium current in ventricular myocytes. Pharmacol Res 2002;45:141–6.
[32] Choi J-S, Hahn SJ, Rhie D-J, Yoon S-H, Jo Y-H, Kim M-S. Mechanism of fluoxetine block of cloned voltage-activated potassium channel Kv1.3. J Pharmacol Exp Ther 1999;291:1–6.
[33] Amsterdam J, Brunswick D, Mendels J. The clinical application of tricyclic antidepressant pharmacokinetics and plasma levels. Am J Psychiatry 1980;137:653–63.
[34] Baldessarini RJ. Drugs and the treatment of psychiatric disorders. Depression and anxiety disorders. In: Hardman JG, Limbird LE, editors. Goodman & Gilman's, the pharmacological basis of therapeutics. 10th ed. New York: McGraw-Hill; 2001. p. 447–83.