



## Role of $\text{Ca}^{2+}$ -activated $\text{K}^+$ channels and $\text{Na}^+, \text{K}^+$ -ATPase in prostaglandin $\text{E}_1$ - and $\text{E}_2$ -induced inhibition of the adrenergic response in human vas deferens

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### ABSTRACT

We studied the role of  $\text{K}^+$  channels and  $\text{Na}^+, \text{K}^+$ -ATPase in the presynaptic inhibitory effects of prostaglandin  $\text{E}_1$  ( $\text{PGE}_1$ ) and  $\text{PGE}_2$  on the adrenergic responses of human vas deferens. Furthermore, we determined the effects of increasing extracellular  $\text{K}^+$  concentrations ( $[\text{K}^+]_o$ ) and inhibition of  $\text{Na}^+, \text{K}^+$ -ATPase on neurogenic and norepinephrine-induced contractile responses. Ring segments of the epididymal part of the vas deferens were taken from 45 elective vasectomies and mounted in organ baths for isometric recording of tension. The neuromodulatory effects of PGEs were tested in the presence of  $\text{K}^+$  channel blockers.  $\text{PGE}_1$  and  $\text{PGE}_2$  ( $10^{-8}$  to  $10^{-6}$  M) induced inhibition of adrenergic contractions. The presence of tetraethylammonium ( $10^{-3}$  M), charybdotoxin ( $10^{-7}$  M), or iberiotoxin ( $10^{-7}$  M), prevented the inhibitory effects of  $\text{PGE}_1$  and  $\text{PGE}_2$  on the adrenergic contraction. Both glibenclamide ( $10^{-5}$  M) and apamin ( $10^{-6}$  M) failed to antagonize  $\text{PGE}_1$  and  $\text{PGE}_2$  effects. Raising the  $[\text{K}^+]_o$  from 15.8 mM to 25.8 mM caused inhibition of the neurogenic contractions. Ouabain at a concentration insufficient to alter the resting tension ( $10^{-6}$  M) increased contractions induced by electrical stimulation but did not alter the contractions to norepinephrine. The inhibition of neurogenic responses induced  $\text{PGE}_1$ ,  $\text{PGE}_2$  and increased extracellular concentration of  $\text{K}^+$  was almost completely prevented by ouabain ( $10^{-6}$  M). The results demonstrate that  $\text{PGE}_1$  and  $\text{PGE}_2$  inhibit adrenergic responses by a prejunctional mechanism that involves the activation of large-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels and  $\text{Na}^+, \text{K}^+$ -ATPase.

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### 1. Introduction

Prostaglandins are a family of biologically lipid acids synthesized by cyclooxygenase from a common precursor, arachidonic acid. Previous studies have indicated that prostaglandin  $\text{E}_1$  ( $\text{PGE}_1$ ) and  $\text{PGE}_2$ , and other prostanoids, influence adrenergic neurotransmission in the vas deferens from several species [1–4]. In human vas deferens, the motor innervation is mainly noradrenergic [5,6] and  $\text{PGE}_1$  and  $\text{PGE}_2$  have been reported to inhibit neurogenic contractions by decreasing the release of norepinephrine from sympathetic nerve endings [7]. The mechanisms whereby  $\text{PGE}_1$  and  $\text{PGE}_2$  modify neurotransmitter release have received much attention but remain poorly understood. Prostaglandins of the E

series inhibit transmitter release by an action on stimulus-secretion coupling and more specifically on the availability of  $\text{Ca}^{2+}$  for the release mechanism [8].

$\text{K}^+$  channels modulate the adrenergic contractile responses in human vas deferens [9] and several agents have been shown to inhibit neurotransmitter release from nerve endings through a mechanism that involves the opening of prejunctional  $\text{K}^+$  channels, membrane hyperpolarization and reduction in  $\text{Ca}^{2+}$  influx via voltage-activated  $\text{Ca}^{2+}$  channels [10,11]. In vas deferens, it has been demonstrated that  $\text{K}^+$  channels are involved in the prejunctional inhibitory effects of atrial natriuretic factor [12],  $\alpha_2$ -adrenoceptor agonists [13] and sildenafil, an inhibitor of phosphodiesterase 5 [14].

Activation of  $\text{K}^+$  channels stimulates cellular  $\text{K}^+$  efflux [15]. In vascular smooth muscle, it has been demonstrated that an increased extracellular  $\text{K}^+$  concentration ( $[\text{K}^+]_o$ ) evokes rapid hyperpolarization and relaxation [16] by stimulating an ouabain-sensitive  $\text{Na}^+, \text{K}^+$ -ATPase [17]. In rat vas deferens, increased extracellular  $\text{K}^+$  concentrations lower than those necessary to induce contraction of the smooth muscle inhibit electrically

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induced contractions [18]. A role for increased  $[K^+]_o$  and  $Na^+,K^+$ -ATPase in modulation of adrenergic neurotransmission in the human vas deferens has not yet been investigated. Therefore, the present study was designed to examine the effects of increasing  $[K^+]_o$  and  $Na^+,K^+$ -ATPase on adrenergic contractions of human vas deferens and the contribution of  $K^+$  channels and  $Na^+,K^+$ -ATPase to the inhibitory effects of  $PGE_1$  and  $PGE_2$  on adrenergic neurotransmission.

## 2. Material and methods

Segments (15–20 mm long) of the epididymal part of the vas deferens were taken from 45 healthy men without previous history of psychiatric or physical illness, medication use, or substance abuse (mean age, 39, range 30–44 years) who were sterilized by elective vasectomy. The study was approved by the Human Ethics Committee of our institution and informed consent was obtained from each subject before the study. The specimens were placed in chilled isotonic NaCl, and were divided into ring preparations 3–4 mm long.

### 2.1. Isolated vas deferens preparation

Ring preparations were suspended between two L-shaped stainless steel pins. One pin was fixed to the organ bath wall while the other was connected to a strain gauge (Grass FT03, Grass Instruments Division Astromed, Inc., West Warwick, RI, USA). Changes in isometric force were recorded by use of Chart v3.4/s software and a MacLab/8e data acquisition system (ADInstruments, East Sussex, UK). Each preparation was set up in a 4-ml bath containing modified Krebs–Henseleit solution of the following millimolar composition: NaCl, 115; KCl, 4.6;  $KH_2PO_4$  1.2;  $MgCl_2 \cdot 6H_2O$ , 1.2;  $CaCl_2$ , 2.5;  $NaHCO_3$ , 25; glucose, 11.1; and disodium EDTA, 0.01. The solution was equilibrated with 95%  $O_2$  and 5%  $CO_2$ . The preparations were allowed to equilibrate for 1-h and during this time tension was adjusted to a final tension of 19.6 mN.

Electrical field stimulation was provided by a Grass S88 stimulator (Grass Instruments Division Astromed, Inc., West Warwick, RI, USA) via two platinum electrodes positioned on each side and parallel to the axis of the ring. Single square wave pulses (0.25 ms pulse duration, 20 Hz, at a supramaximal voltage of  $20 V cm^{-1}$ ) were used. The train duration was 5 s and the stimulation interval 180 s. The stimulation parameters used elicit contractile responses that are abolished by tetrodotoxin ( $10^{-6}$  M) or prazosin ( $10^{-6}$  M) [19].

### 2.2. Experimental procedure

To study the neuromodulatory action of prostaglandins, when electrically induced phasic contractions were stable (after 15–20 min),  $PGE_1$  or  $PGE_2$  ( $10^{-8}$  to  $10^{-6}$  M) were added cumulatively to the preparations and the effects of electrical field stimulation were recorded. To examine the role of  $K^+$  channel activation in the effects of  $PGE_1$  and  $PGE_2$  on electrical field stimulation induced contractions, concentration–response curves to  $PGE_1$  and  $PGE_2$  were established in the presence of one of the following inhibitors: TEA ( $10^{-3}$  M), a nonspecific  $K^+$  channel blocker [20], glibenclamide ( $10^{-5}$  M), a selective blocker of ATP-sensitive  $K^+$  ( $K_{ATP}$ ) channels [21], charybdotoxin ( $10^{-7}$  M), an inhibitor of  $Ca^{2+}$ -activated  $K^+$  ( $K_{Ca}$ ) channels of both large-conductance ( $K_{Ca1.1}$ ) [22] and intermediate-conductance ( $K_{Ca3.1}$ ) [23], iberiotoxin ( $10^{-7}$  M), an inhibitor of  $K_{Ca1.1}$  channels [24], or apamin ( $10^{-6}$  M), an inhibitor of  $K_{Ca}$  of small-conductance ( $K_{Ca2}$ ) channels [25].

Concentration–response curves to norepinephrine were obtained in a cumulative manner in the absence (control) and in the presence of  $PGE_1$  ( $10^{-6}$  M) or  $PGE_2$  ( $10^{-6}$  M) from separate preparations.

$K^+$  channels activation stimulates  $K^+$  efflux [15]. As increases in the  $[K^+]_o$  evoke rapid vascular smooth muscle relaxation and hyperpolarization [16], we studied the adrenergic responses in the presence of increased  $[K^+]_o$  (10.8–25.8 mM) to check the possibility that increasing  $[K^+]_o$  may inhibit adrenergic contractions. At the end of each series, the physiological concentration of  $K^+$  was restored by washing out with Krebs–Henseleit solution and electrical stimulation was repeated to confirm the reversibility of the effects of high  $K^+$  concentrations on neurogenic response.

To determine whether  $Na^+,K^+$ -ATPase activity is involved in the adrenergic responses of the human vas deferens, the contractions to electrical field stimulation and norepinephrine were recorded before and 15 min after the addition of ouabain ( $10^{-7}$  to  $10^{-6}$  M), a  $Na^+,K^+$ -ATPase inhibitor.

In another group of experiments, the influence of  $Na^+,K^+$ -ATPase on the  $PGE_1$ -,  $PGE_2$ - or increasing  $K^+$  concentrations-induced inhibition of contractions evoked by electrical field stimulation was examined by exposing vas deferens rings to ouabain ( $10^{-6}$  M) for 15 min before the addition of  $PGE_1$  ( $10^{-8}$  to  $3 \times 10^{-6}$  M),  $PGE_2$  ( $10^{-8}$  to  $3 \times 10^{-6}$  M) or  $[K^+]_o$  (10.8–25.8 mM) and the responses to electrical stimulation were recorded.

### 2.3. Drugs

The following drugs were used: norepinephrine hydrochloride, prazosin hydrochloride, tetrodotoxin, prostaglandin  $E_1$  ( $PGE_1$ ), prostaglandin  $E_2$  ( $PGE_2$ ), tetraethylammonium bromide (TEA), charybdotoxin, iberiotoxin, apamin, glibenclamide and ouabain (Sigma Chemical Co., St. Louis, MO, USA). All drugs were dissolved in Krebs solution, except for glibenclamide and ouabain which were dissolved initially in dimethyl sulfoxide and further diluted in Krebs solution to the proper final concentration. Stock solutions of the drugs were freshly prepared every day, and kept on ice throughout the experiment.

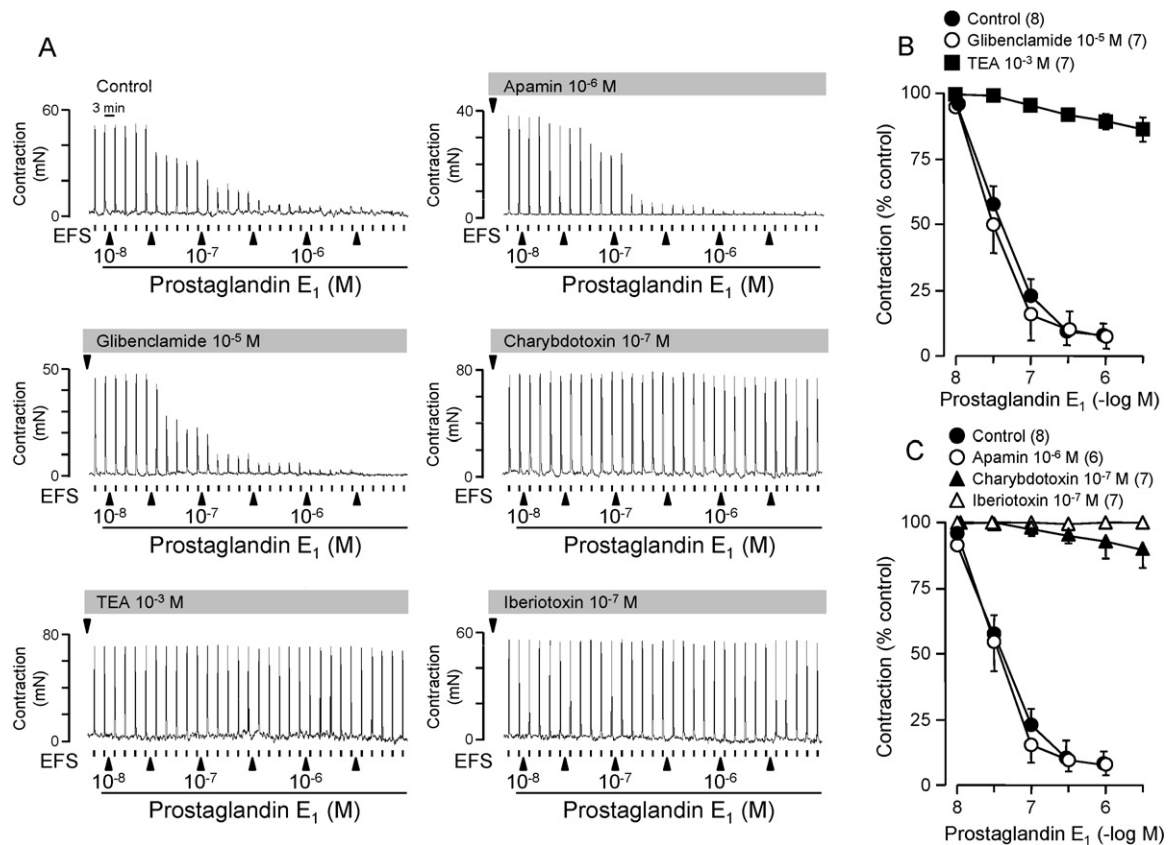
### 2.4. Data analysis

All values are expressed as means  $\pm$  S.E.M. Contractions are reported as absolute values (mN) or as percentages of control responses.  $pD_2$  values (negative logarithm of the molar concentration at which half-maximum contraction occurs) were determined from individual concentration–response curves by non-linear regression analysis. The responses obtained in each subject were averaged to yield a single value. Differences between untreated and treated groups were assessed by one-way analysis of variance (ANOVA), and then Bonferroni's test was performed. Statistical significance was accepted at  $P < 0.05$ .

## 3. Results

Electrical field stimulation (EFS) induced phasic contraction in segments of the vas deferens which was abolished by tetrodotoxin ( $10^{-6}$  M) and prazosin ( $10^{-6}$  M), thus indicating that the smooth muscle contraction was due to the release of norepinephrine from adrenergic nerves acting on  $\alpha_1$  adrenoceptors (results not shown).

Fig. 1A shows representative tracings of the effects of  $PGE_1$  ( $10^{-8}$  to  $10^{-6}$  M) on EFS-evoked contractions of the vas deferens rings in the absence (control) and in the presence of  $K^+$  channel blockers and Fig. 1B and C summarizes the results from all the experiments.  $PGE_1$  induced concentration-dependent inhibition of EFS-evoked contractions with a  $pD_2$  value of  $7.35 \pm 0.10$  (Fig. 1B and C). Apamin ( $10^{-6}$  M) and glibenclamide ( $10^{-5}$  M) did not modify the inhibitory effect of  $PGE_1$  on neurogenic contractions whereas charybdotoxin ( $10^{-7}$  M), TEA ( $10^{-3}$  M) and iberiotoxin ( $10^{-7}$  M) reduced significantly the inhibitory effects of  $PGE_1$ . The effects of  $PGE_2$



**Fig. 1.** (A) Representative tracings showing the inhibitory effect of prostaglandin E<sub>1</sub> on electrical field stimulation (EFS)-evoked contractions of human vas deferens rings in the absence and in the presence of K<sup>+</sup> channel blockers. (B) and (C) summarize the results from all the experiments. Results were calculated as the percentage of the contractile response elicited by EFS (0.25 ms pulse duration, 20 Hz, at a supramaximal voltage of 20 V cm<sup>-1</sup>, train duration 5 s) before the addition of prostaglandin E<sub>1</sub>. Values are presented as the mean ± S.E.M. Numbers in parentheses are the number of subjects in each group.

on neurogenic contractions and the inhibition of these effects by K<sup>+</sup> channel blockers were similar to those observed with PGE<sub>1</sub> (Fig. 2).

Fig. 3A and B shows that the increase in [K<sup>+</sup>]<sub>o</sub> in the Krebs-Henseleit solution caused concentration-dependent inhibition of the EFS-induced contractions with a maximum inhibitory effect of 96%. The inhibition was reversible after washout of the excess of K<sup>+</sup>.

Ouabain (10<sup>-7</sup> to 10<sup>-6</sup> M) had no significant effect on the basal tone of the preparations but increased contractile responses to EFS

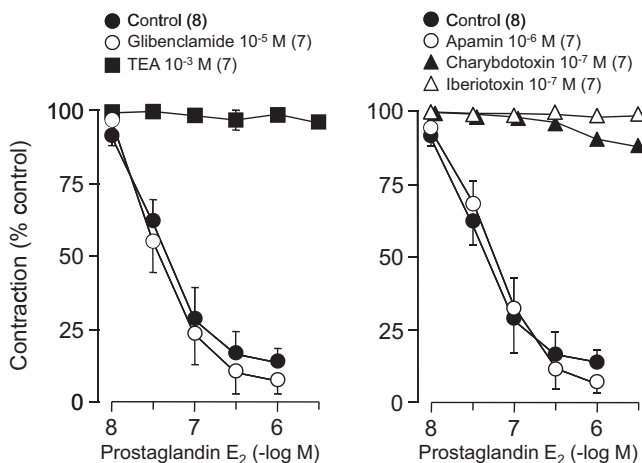
(Fig. 3C and D). Ouabain (10<sup>-6</sup> M) counteracted the inhibitory effects of PGE<sub>1</sub>, PGE<sub>2</sub> and increasing [K<sup>+</sup>]<sub>o</sub> on EFS-induced contractions (Fig. 4).

Cumulative addition of norepinephrine (10<sup>-6</sup> to 3 × 10<sup>-4</sup> M) induced repetitive phasic, concentration-dependent contractions with a pD<sub>2</sub> value of 5.01 ± 0.10. Pretreatment with PGE<sub>1</sub> (10<sup>-6</sup> M) or PGE<sub>2</sub> (10<sup>-6</sup> M) did not affect the contractions induced by norepinephrine (Table 1). Moreover, pretreatment with either ouabain (10<sup>-6</sup> M) or increasing [K<sup>+</sup>]<sub>o</sub> to 25.8 mM were without effect on norepinephrine-induced contractions (Fig. 5 and Table 1).

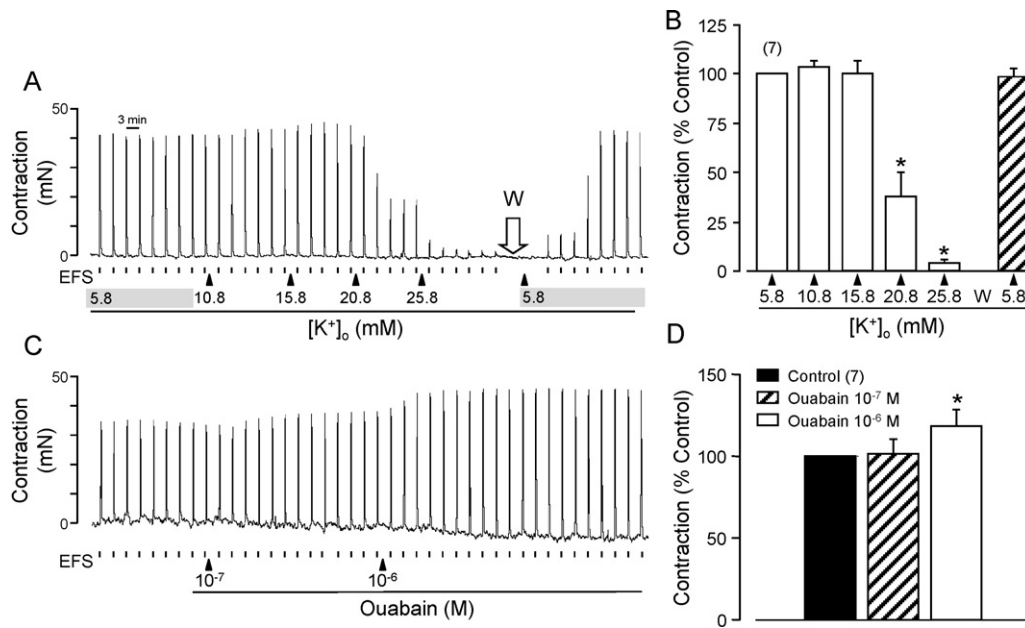
#### 4. Discussion

The present study demonstrates a role for K<sup>+</sup> channels and Na<sup>+</sup>,K<sup>+</sup>-ATPase in the prejunctional inhibitory effects of PGE<sub>1</sub> and PGE<sub>2</sub> on adrenergic responses of human vas deferens. It has been demonstrated that PGE<sub>1</sub> and PGE<sub>2</sub> selectively depress neurogenic contractions in human vas deferens without modifying the contractions to norepinephrine [7]. The lack of effect of PGE<sub>1</sub> and PGE<sub>2</sub> on norepinephrine-induced contractions suggests that postjunctional inhibitory mechanisms are not involved in the neuromodulatory action of these prostaglandins. The inhibitory effect on neurogenic contractions has been attributed to decreased norepinephrine release from nerve endings in the human vas deferens [7].

The mechanisms whereby PGE<sub>1</sub> and PGE<sub>2</sub> induce inhibition of neurotransmitter release remain poorly understood. Several agents could inhibit neurotransmitter release from nerve endings in vas deferens through a mechanism that involves activation of K<sup>+</sup> channels. In human vas deferens, charybdotoxin-sensitive, iberiotoxin-insensitive, K<sup>+</sup> channels modulate the adrenergic contrac-



**Fig. 2.** Graphical representation of the inhibition of EFS-induced contractions by increasing concentrations of prostaglandin E<sub>2</sub> in the absence (control) and in the presence of K<sup>+</sup> channel blockers. Results were calculated as the percentage of the contractile response elicited by EFS before the addition of prostaglandin E<sub>2</sub>. Values are presented as the mean ± S.E.M. Numbers in parentheses are the number of subjects in each group.



**Fig. 3.** (A) Representative tracing and (B) bar graph showing the inhibitory effects of increasing extracellular  $K^+$  concentration ( $[K^+]_o$ ) from 5.8 to 25.8 mM on electrical field stimulation (EFS)-induced contractions. After wash out (W) with fresh Krebs–Henseleit solution ( $[K^+]_o = 5.8$  mM), the mean amplitude of contractions induced by EFS was reverted to initial conditions. (C) Representative tracing and (D) bar graph showing the contractile responses to EFS in the absence (control) and in the presence of ouabain ( $10^{-7}$  to  $10^{-6}$  M). Results in (B) and (D) were calculated as the percentage of the contractile response elicited by EFS before the addition of  $K^+$  or ouabain. Values are mean  $\pm$  S.E.M. Numbers in parentheses are the number of subjects in each group. \* $P < 0.05$  versus control.

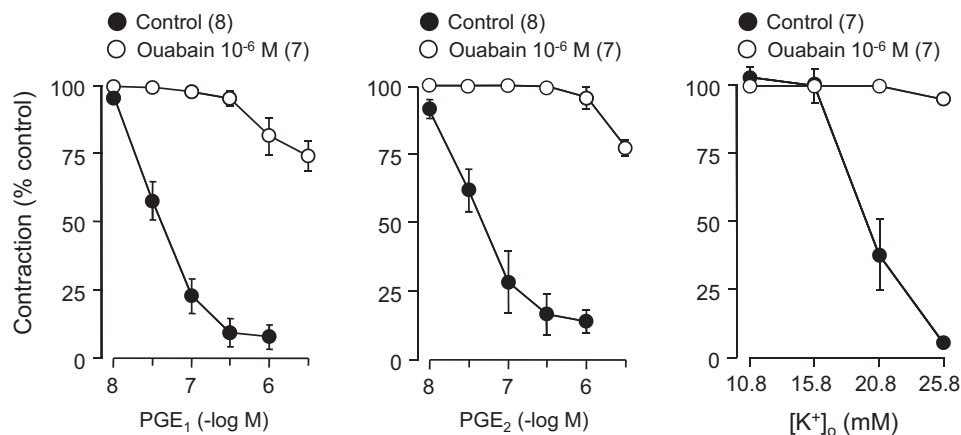
tile response by interfering with  $Ca^{2+}$  entry through dihydropyridine  $Ca^{2+}$  channels [9]. Furthermore, it has been shown that  $K^+$  channels are involved in the prejunctional inhibitory effects of atrial natriuretic factor in the rabbit isolated vas deferens [12] and in the  $\alpha_2$ -adrenoceptor-mediated inhibition in rat vas deferens [13]. In the human vas deferens, sildenafil, an inhibitor of phosphodiesterase 5, inhibits adrenergic contractions through activation of presynaptic  $K_{Ca1.1}$  channels [14].

Glibenclamide has been shown to block  $K_{ATP}$  channels [21] with an  $IC_{50}$  in the micromolar range for smooth muscle preparations [26]. In rat vas deferens, the inhibitory action of calcitonin-gene-related peptide (CGRP) on neurotransmission was at least in part mediated by the activation of  $K_{ATP}$  channels in sympathetic nerve terminals as shown by the prevention of the neuromodulatory effects of CGRP in the presence of glibenclamide  $10^{-5}$  M [27]. Our results in human vas deferens demonstrate that glibenclamide at concentration as high as  $10^{-5}$  M did not modify the inhibition

induced by PGEs on adrenergic responses. These results rule out the involvement of  $K_{ATP}$  channels activation in the inhibitory effects of PGEs on neurogenic contractions of the human vas deferens.

On the other hand, we tested the effects of TEA, a nonspecific  $K^+$  channel blocker [20] on the neuromodulatory effects of PGE<sub>1</sub> and PGE<sub>2</sub>. It is accepted that blockade with  $10^{-3}$  M TEA is sufficient to almost fully inhibit  $K_{Ca1.1}$  channels [28]. In our experiments millimolar concentrations of TEA prevented the inhibitory effects of PGE<sub>1</sub> and PGE<sub>2</sub> on neurogenic contractions, suggesting a role of  $K_{Ca}$  channels in this effect.

To further characterize the member of  $K_{Ca}$  channel group involved in the neuromodulatory effects of PGEs we used more selective blockers of  $K_{Ca}$  channels. Apamin has proved to be extremely valuable as a highly specific blocker for which the only known receptors are the  $K_{Ca}$  channels of small conductance ( $K_{Ca2}$ ) [29]. In our experiments, apamin  $10^{-6}$  M, a concentration expected to block completely all subtypes of  $K_{Ca2}$  channels, failed to alter the



**Fig. 4.** Inhibition of electrical field stimulation (EFS)-induced contractions by prostaglandin E<sub>1</sub> (PGE<sub>1</sub>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and increasing extracellular  $K^+$  concentration ( $[K^+]_o$ ) from 10.8 to 25.8 mM in the absence (control) and in the presence of ouabain ( $10^{-6}$  M). Results were calculated as the percentage of the contractile response elicited by EFS before the addition of PGE<sub>1</sub>, PGE<sub>2</sub> or  $K^+$ . Values are mean  $\pm$  S.E.M. Numbers in parentheses are the number of subjects in each group.



**Table 1**

pD<sub>2</sub> values and maximal contractions elicited by norepinephrine alone (control) and in the presence of prostaglandin E<sub>1</sub> (PGE<sub>1</sub>), PGE<sub>2</sub>, ouabain or increased extracellular K<sup>+</sup> concentration ([K<sup>+</sup>]<sub>o</sub>).

Norepinephrine	pD <sub>2</sub> ± S.E.M.	Maximum contraction (mN ± S.E.M.)
Control (n = 7)	5.01 ± 0.10	49.7 ± 7.9
PGE <sub>1</sub> (10 <sup>-6</sup> M) (n = 5)	5.11 ± 0.09	48.6 ± 5.3
PGE <sub>2</sub> (10 <sup>-6</sup> M) (n = 5)	5.02 ± 0.09	47.5 ± 7.4
Ouabain (10 <sup>-6</sup> M) (n = 5)	4.95 ± 0.14	49.5 ± 8.3
[K <sup>+</sup> ] <sub>o</sub> = 25.8 mM (n = 5)	5.07 ± 0.10	47.9 ± 9.1

Values are means ± S.E.M. n, number of subjects.

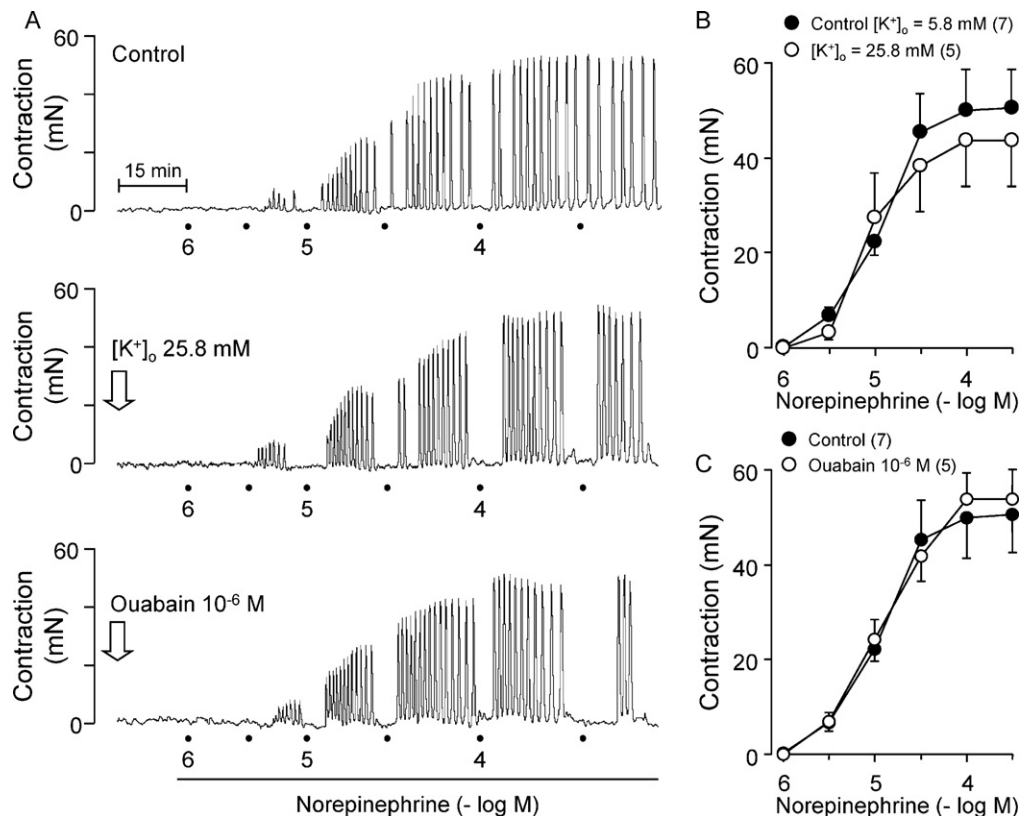
inhibition caused by PGE<sub>1</sub> and PGE<sub>2</sub> on neurogenic contractions in human vas deferens. These data indicate that K<sub>Ca</sub>2 channels are not involved in the neuromodulatory effects of PGE<sub>1</sub> and PGE<sub>2</sub>.

Charybdotoxin, a blocker of both K<sub>Ca</sub>1.1 and K<sub>Ca</sub>3.1 channels [22,23], prevented the inhibitory effects of PGE<sub>1</sub> and PGE<sub>2</sub> on sympathetic contractions of the human vas deferens. These results demonstrate that the K<sup>+</sup> channels involved are sensitive to charybdotoxin. Therefore, we tested the effect of iberiotoxin, a selective blocker of K<sub>Ca</sub>1.1 channels [24], on the effects of PGE<sub>1</sub> and PGE<sub>2</sub> on adrenergic contractions. The marked effect of iberiotoxin in the present study provides evidence for K<sub>Ca</sub>1.1 channels as a significant component of PGE<sub>1</sub> and PGE<sub>2</sub> inhibitory effects on neurogenic contractions of the human vas deferens.

A previous study in rat vas deferens demonstrated that increasing [K<sup>+</sup>]<sub>o</sub> at concentrations lower than those necessary to induce contraction of the smooth muscle, completely inhibited the electrically induced contractions [18], an effect attributed to a desensitization of the rat vas deferens to electrically induced

contractions. Studies of vascular smooth muscle showed that increased [K<sup>+</sup>]<sub>o</sub> in the range 6–16 mM produce hyperpolarization and vasodilatation by stimulating an ouabain-sensitive Na<sup>+</sup>,K<sup>+</sup>-ATPase [17]. In guinea-pig vas deferens relatively high concentrations of ouabain, an inhibitor of Na<sup>+</sup>,K<sup>+</sup>-ATPase, is reported to cause a gradual increase in the norepinephrine release from the peripheral adrenergic neurons [30] and an increase in the sensitivity to norepinephrine [31]. Particularly for the vas deferens, species is thought to play an important role in Na<sup>+</sup>,K<sup>+</sup>-ATPase activity and function [31]. However, the effects of inhibition of Na<sup>+</sup>,K<sup>+</sup>-ATPase on adrenergic responses of human vas deferens have not been investigated. As the resting tension of human vas deferens was not changed by ouabain in our experiments, we suggest that Na<sup>+</sup>,K<sup>+</sup>-ATPase is not normally active in the relaxed vas deferens smooth muscle. Furthermore, ouabain had no effect on norepinephrine-mediated contractions at concentration of 10<sup>-6</sup> M, but increased neurogenic contractions. These results indicate that the enhanced neurogenic contractions induced by ouabain involves the inhibition of Na<sup>+</sup>,K<sup>+</sup>-ATPase activity of the adrenergic nerve endings.

Increasing [K<sup>+</sup>]<sub>o</sub> over the range that inhibits electrical field stimulation-induced adrenergic contractions should also stimulate the Na<sup>+</sup>,K<sup>+</sup>-ATPase by increasing the amount of extracellular K<sup>+</sup> that binds to the pump [32]. In human vas deferens, ouabain blocks K<sup>+</sup>-induced inhibition of adrenergic contractions, indicating that increasing [K<sup>+</sup>]<sub>o</sub> stimulates the Na<sup>+</sup>,K<sup>+</sup>-ATPase in the range that inhibits adrenergic contractions. Because Na<sup>+</sup>,K<sup>+</sup>-ATPase activity is ultimately responsible for most of the resting membrane potential, the ability of increased extracellular K<sup>+</sup> to regulate adrenergic contractions could in part be due to its effect on Na<sup>+</sup>,K<sup>+</sup>-ATPase activity. There are two effects of the pump on membrane potential: (i) an electrogenic effect, due to the 3Na<sup>+</sup>/2K<sup>+</sup> exchange (this is the



**Fig. 5.** (A) Recordings illustrating the contractile effects of norepinephrine in the absence (control, extracellular K<sup>+</sup> concentration [K<sup>+</sup>]<sub>o</sub> = 5.8 mM) and in the presence of [K<sup>+</sup>]<sub>o</sub> of 25.8 mM or ouabain (10<sup>-6</sup> M). Concentration–response curves for norepinephrine in the absence (control, [K<sup>+</sup>]<sub>o</sub> = 5.8 mM) and in the presence of (B) [K<sup>+</sup>]<sub>o</sub> of 25.8 mM, and (C) in the presence of ouabain (10<sup>-6</sup> M). Values are mean ± S.E.M. Numbers in parentheses are the number of subjects in each group. \*P < 0.05 versus control.

smaller, but more rapid effect) and (ii) by maintaining the transmembrane  $K^+$  gradient the membrane potential is created (this is the more powerful, but slower effect). Our results suggest that in the inhibitory effects of  $K^+$  on the adrenergic contractions of human vas deferens only the first effect is relevant because in our experimental conditions it is not possible to increase the transmembrane  $K^+$  gradient by increasing  $[K^+]_o$ .

However, involvement of other mechanisms/pathways cannot be excluded. Recently, it has been demonstrated the proximity and functional interaction between the  $K_{Ca}1.1$  channel and  $Na^+,K^+$ -ATPase [33]. This interplay is suggested to depend on the proper microenvironment. Previous studies demonstrated that inhibition of  $Na^+,K^+$ -ATPase and subsequent accumulation of intracellular  $Na^+$  decreases  $K_{Ca}1.1$  current [33,34]. Another possibility is that inhibition of  $Na^+,K^+$ -ATPase by ouabain leads to an accumulation of  $Na^+$  close to the microdomains harboring  $K_{Ca}1.1$  channels, which inhibits the channel function.

Furthermore, ouabain blocked the inhibitory effects of  $PGE_1$  and  $PGE_2$  on sympathetic contractions in human vas deferens, suggesting that activation of  $Na^+,K^+$ -ATPase plays an important role in the presynaptic inhibition of the norepinephrine release induced by these prostaglandins. Consistent with our data, an acute stimulatory effect of  $PGE_1$  and  $PGE_2$  on  $Na^+,K^+$ -ATPase activity was observed in rabbit renal proximal tubule cells and cortical collecting tubule of the nephron [35,36].

In rat vas deferens it has been demonstrated that  $PGE_2$  is released from the epithelium in response to ATP stimulation and it is responsible for mediating the membrane hyperpolarization and inhibition of contraction of smooth muscle cells by activating cAMP-dependent  $K^+$  channels [37]. However, our results show that  $PGE_2$  inhibited neurogenic contractions but did not exert inhibitory effects on norepinephrine-induced contractions, indicating that  $PGE_2$  acts directly on sympathetic nerve endings.

An important function of the vas deferens is the transport of spermatozoa from the epididymis for inclusion in the semen. Since  $\alpha_1$ -adrenoceptors activation by norepinephrine is required for normal contraction of the vas deferens and consequent sperm ejaculation [38], it seems likely that increased formation of  $PGE_2$  in physiological and pathological conditions such as inflammation [39], may cause aperistalsis of the adrenergically innervated vas deferens by activating  $K_{Ca}1.1$  channels and  $Na^+,K^+$ -ATPase and impairment of male fertility. Although the present studies provide evidence that activation of presynaptic  $K_{Ca}1.1$  channels inhibits adrenergic contractions of vas deferens, future studies are necessary to elucidate the potential therapeutic modification of vas deferens motility, via pharmacologic intervention of these channels.

The findings of the present study support the view that the presynaptic inhibitory effect of  $PGE_1$  and  $PGE_2$  is mediated via activation of large-conductance  $K_{Ca}$  channels. Increasing  $[K^+]_o$  induces reversible inhibition of adrenergic vas deferens contractions. These inhibitory effects on neurogenic contractions of human vas deferens are mediated by activation of  $Na^+,K^+$ -ATPase.

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## References

- [1] Hedqvist P, von Euler US. Prostaglandin-induced neurotransmission failure in the field-stimulated, isolated vas deferens. *Neuropharmacology* 1972;11:177–87.
- [2] Illes P, Ronai A, Knoll J. Inhibition of neuromuscular transmission by prostaglandin E1 in the circular muscle of the guinea-pig vas deferens. *Med Biol* 1976;54:400–5.
- [3] Miller MR, Johnson FC, Poyser NL. Endogenous prostaglandin formation in the field-stimulated guinea-pig vas deferens: comparison of the inhibitory effects of indomethacin and NS-398. *Prostaglandins Leukot Essent Fatty Acids* 1999;61:363–8.
- [4] McKay AM, Poyser NL. A comparison of the effects of exogenous and endogenous prostaglandins on fast and slow contractions of field-stimulated guinea-pig vas deferens. *Br J Pharmacol* 1995;116:2679–84.
- [5] Birmingham AT. The human isolated vas deferens: its response to electrical stimulation and to drugs. *Br J Pharmacol* 1968;34:692P–3P.
- [6] Hedlund H, Andersson KE, Larsson B. Effect of drugs interacting with adrenoceptors and muscarinic receptors in the epididymal and prostatic parts of the human isolated vas deferens. *J Auton Pharmacol* 1985;5:261–70.
- [7] Holmquist F, Hedlund H, Andersson KE. Pre- and postjunctional effects of some prostanooids in human isolated vas deferens. *Am J Physiol* 1991;260:R792–7.
- [8] Malik KU, Sehic E. Prostaglandins and the release of the adrenergic transmitter. *Ann N Y Acad Sci* 1990;604:222–36.
- [9] Medina P, Segarra G, Mauricio MD, Vila JM, Chuan P, Lluch S. Modulation of adrenergic responses of human vas deferens by  $K^+$  channel inhibitors. *Urology* 2010;76:1518–612.
- [10] North RA, Williams JT, Surprenant A, Christie MJ. Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. *Proc Natl Acad Sci USA* 1987;84:5487–91.
- [11] Miller RJ. Receptor-mediated regulation of calcium channels and neurotransmitter release. *FASEB J* 1990;4:3291–9.
- [12] Kanwal S, Trachte GJ. Potassium channel inhibitors attenuate neuromodulatory effects of atrial natriuretic factor in the rabbit isolated vas deferens. *J Pharmacol Exp Ther* 1994;268:117–23.
- [13] Docherty JR, Brady G. Prejunctional actions of  $K^+$  channel blockers in rat vas deferens. *Eur J Pharmacol* 1995;287:287–93.
- [14] Medina P, Segarra G, Torondel B, Chuan P, Domenech C, Vila JM, et al. Inhibition of neuroeffector transmission in human vas deferens by sildenafil. *Br J Pharmacol* 2000;131:871–4.
- [15] Gordon JL, Martin W. Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of  $^{86}Rb$  efflux from isolated endothelial cells. *Br J Pharmacol* 1983;79:531–41.
- [16] Edwards FR, Hirst GD. Inward rectification in submucosal arterioles of guinea-pig ileum. *J Physiol* 1988;404:437–54.
- [17] Webb RC, Bohr DF. Potassium-induced relaxation as an indicator of  $Na^+-K^+$  ATPase activity in vascular smooth muscle. *Blood Vessels* 1978;15:198–207.
- [18] Banchelli G, Raimondi L, Ghelardini C, Pirisino R, Bertini V, De MA, et al. Benzylamine-related compounds stimulate rat vas deferens neurotransmission and potentiate memory in the mouse acting as potassium channel blockers. *Pharmacol Res* 2000;41:151–62.
- [19] Medina P, Segarra G, Chuan P, Domenech C, Vila JM, Aldasoro M, et al. Vasopressin receptors involved in adrenergic neurotransmission in the circular muscle of the human vas deferens. *Eur J Pharmacol* 1998;355:41–9.
- [20] Benham CD, Bolton TB, Lang RJ, Takewaki T. The mechanism of action of  $Ba^{2+}$  and TEA on single  $Ca^{2+}$ -activated  $K^+$ -channels in arterial and intestinal smooth muscle cell membranes. *Pflügers Arch* 1985;403:120–7.
- [21] Sturgess NC, Ashford ML, Cook DL, Hales CN. The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* 1985;2:474–5.
- [22] Miller C, Moczydowski E, Latorre R, Phillips M. Charybdotoxin, a protein inhibitor of single  $Ca^{2+}$ -activated  $K^+$  channels from mammalian skeletal muscle. *Nature* 1985;313:316–8.
- [23] Brugnara C, Armsby CC, De FL, Crest M, Euclaire MF, Alper SL.  $Ca^{2+}$ -activated  $K^+$  channels of human and rabbit erythrocytes display distinctive patterns of inhibition by venom peptide toxins. *J Membr Biol* 1995;147:71–82.
- [24] Galvez A, Gimenez-Gallego G, Reuben JP, Roy-Contancin L, Feigenbaum P, Kaczorowski GJ, et al. Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. *J Biol Chem* 1990;265:11083–90.
- [25] Blatz AL, Magleby KL. Single apamin-blocked  $Ca^{2+}$ -activated  $K^+$  channels of small conductance in cultured rat skeletal muscle. *Nature* 1986;323:718–20.
- [26] Winquist RJ, Heaney LA, Wallace AA, Baskin EP, Stein RB, Garcia ML, et al. Glyburide blocks the relaxation response to BRL 34915 (cromakalim), minoxidil sulfate and diazoxide in vascular smooth muscle. *J Pharmacol Exp Ther* 1989;248:149–56.
- [27] Huang Y, Lau CW, Ho IH. Involvement of ATP-sensitive  $K^+$  channels in the inhibitory effect of calcitonin gene-related peptide on neurotransmission in rat vas deferens. *Eur J Pharmacol* 1997;327:209–14.
- [28] Kaczorowski GJ, Knaus HG, Leonard RJ, McManus OB, Garcia ML. High-conductance calcium-activated potassium channels; structure, pharmacology, and function. *J Bioenerg Biomembr* 1996;28:255–67.
- [29] Stocker M.  $Ca^{2+}$ -activated  $K^+$  channels: molecular determinants and function of the SK family. *Nat Rev Neurosci* 2004;5:758–70.
- [30] Nakazato Y, Ohga A, Onoda Y. The effect of ouabain on noradrenaline output from peripheral adrenergic neurones of isolated guinea-pig vas deferens. *J Physiol* 1978;278:45–54.
- [31] Urquilla PR, Westfall DP, Goto K, Fleming WW. The effects of ouabain and alterations in potassium concentration on the sensitivity to drugs and the membrane potential of the smooth muscle of the guinea-pig and rat vas deferens. *J Pharmacol Exp Ther* 1978;207:347–55.

- [32] Glynn IM. Sodium and potassium movements in human red cells. *J Physiol* 1956;134:278–310.
- [33] Tajima N, Itokazu Y, Korpi ER, Somerharju P, Kakela R. Activity of BK<sub>Ca</sub> channel is modulated by membrane cholesterol content and association with Na<sup>+</sup>/K<sup>+</sup>-ATPase in human melanoma IGR39 cells. *J Biol Chem* 2011;286:5624–38.
- [34] Liang GH, Kim MY, Park S, Kim JA, Choi S, Suh SH. Intracellular Na<sup>+</sup> modulates large conductance Ca<sup>2+</sup>-activated K<sup>+</sup> currents in human umbilical vein endothelial cells. *Pflugers Arch* 2008;457:67–75.
- [35] Scherzer P, Wald H, Rubinger D, Popovtzer MM. Indomethacin and sodium retention in the rat: role of inhibition of prostaglandin E<sub>2</sub> synthesis. *Clin Sci (Lond)* 1992;83:307–11.
- [36] Herman MB, Rajkhowa T, Cutuli F, Springate JE, Taub ML. Regulation of renal proximal tubule Na K-ATPase by prostaglandins. *Am J Physiol Renal Physiol* 2010;298:F1222–34.
- [37] Ruan YC, Wang Z, Du JY, Zuo WL, Guo JH, Zhang J, et al. Regulation of smooth muscle contractility by the epithelium in rat vas deferens: role of ATP-induced release of PGE<sub>2</sub>. *J Physiol* 2008;586:4843–57.
- [38] Sanbe A, Tanaka Y, Fujiwara Y, Tsumura H, Yamauchi J, Cotecchia S, et al.  $\alpha_1$ -adrenoceptors are required for normal male sexual function. *Br J Pharmacol* 2007;152:332–40.
- [39] Legler DF, Bruckner M, Uetz-von AE, Krause P. Prostaglandin E<sub>2</sub> at new glance: novel insights in functional diversity offer therapeutic chances. *Int J Biochem Cell Biol* 2010;42:198–201.