

## Regulation of adrenergic nerve-mediated contraction of canine pulmonary artery by K<sup>+</sup> channels

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*Regulation of adrenergic nerve-mediated contraction of canine pulmonary artery by K<sup>+</sup> channels. E. Tagaya, J. Tamaoki, H. Takemura, A. Nagai. ©ERS Journals Ltd 1998.*

**ABSTRACT:** The aim of the present study was to investigate the role of certain subtypes of K<sup>+</sup> channels in nerve-evoked contractions of pulmonary artery *in vitro*.

The lobar or segmental pulmonary arteries were dissected from dogs, cut into ring segments, and the contractile responses to electrical field stimulation (EFS) and noradrenaline were measured under isometric conditions.

Addition of iberiotoxin, a big conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel blocker, and apamin, a small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel blocker, did not change the resting tension but augmented the contractile responses to EFS, so that the electric stimulus frequency required to produce a half-maximal contraction (ES<sub>50</sub>) was decreased from 18.2±3.5 to 7.4±2.3 Hz (p<0.01) and from 16.8±2.2 to 11.4±2.0 Hz (p<0.05), respectively, whereas glibenclamide, an adenosine triphosphate (ATP)-sensitive K<sup>+</sup> channel blocker, had no effect. In contrast, none of the K<sup>+</sup> channel blockers altered the contractile response to noradrenaline. Incubation of tissues with iberiotoxin and apamin increased the release of <sup>3</sup>H-noradrenaline evoked by EFS.

We conclude that big conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels and small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels may play a role in the regulation of adrenergic neurotransmission in the pulmonary artery, probably by inhibiting the exocytotic release of noradrenaline from the adrenergic nerve terminals.

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It is known that K<sup>+</sup> channels are present on smooth muscle cells and may play an important role in the regulation of vascular and airway smooth muscle tone in the respiratory system [1, 2]. To date, various types of K<sup>+</sup> channels have been found, including voltage-dependent K<sup>+</sup> channels, big conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels, small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels, and adenosine triphosphate (ATP)-sensitive K<sup>+</sup> channels, in vascular smooth muscle cells [2–4]. Activation of these channels causes membrane hyperpolarization or repolarization, which in turn inhibits Ca<sup>2+</sup> influx through voltage-dependent Ca<sup>2+</sup> channels and consequently produces vasodilation [5].

With respect to the effects of K<sup>+</sup> channels on autonomic nerve functions, we and others have recently shown that big conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels are operating in the release of acetylcholine from vagal nerve terminals in canine and human bronchi [6, 7], suggesting that big conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels may also modulate cholinergic neurotransmission and, hence, the parasympathetic component of airway smooth muscle tone. However, the role of K<sup>+</sup> channels in the regulation of adrenergic neurotransmission in pulmonary vascular smooth muscle is unknown. Therefore, in the present study, to determine whether K<sup>+</sup> channels are involved in the adrenergic nerve-mediated contraction of pulmonary artery and, if so, what the mechanism of action is, we studied isolated canine pulmonary arterial segments under isometric conditions *in vitro*.

### Materials and methods

#### Preparation of tissues

Mongrel dogs weighing 18–25 kg were anaesthetized with *i.v.* sodium pentobarbital (35 mg·kg<sup>-1</sup>), and intrapulmonary arterial branches were removed. The lobar or segmental arteries were gently dissected free of underlying connective tissues. Care was taken to avoid stretching or touching the inside of the lumen. Ring segments, 4–6 mm in outer diameter and 4 mm in length, were cut from the vessels and mounted in the organ chambers filled with 14 mL Krebs-Henseleit (KH) solution consisting of the following composition: NaCl, 118; KCl, 5.9; MgSO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 2.5; NaH<sub>2</sub>PO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 25.5; and D-glucose, 5.6 mM, which was maintained at 37°C at a pH 7.4 aerated constantly with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The lower end of the tissues was attached to a glass hook at the base of the organ chamber by a loop of silk thread. The upper end was attached in a similar manner to a force displacement transducer (Model TB-652T; Nihon Kohden, Tokyo, Japan) for continuous recording of isometric tension by a pen recorder (Model WT-685G; Nihon Kohden). Each organ chamber was fitted with two rectangular platinum electrodes (6 × 25 mm) placed alongside the tissues for transmural electrical field stimulation (EFS), using biphasic pulses (pulse duration 0.5 ms, supramaximal voltage of 20 mV for 20 s). Our preliminary experiments

showed that pretreatment of tissues with tetrodotoxin ( $10^{-6}$  M) or phentolamine ( $10^{-5}$  M) abolished the contractile responses to EFS at stimulus frequencies of 1–50 Hz, indicating that the contraction was entirely due to the release of noradrenaline from adrenergic nerves.

The arterial rings were allowed to equilibrate in the chamber for 60 min, while they were washed with KH solution every 15 min, and the resting tension was adjusted to  $3 \times g$ . A contractile response was measured as the difference between peak tension developed and resting tension.

#### *Effect of K<sup>+</sup> channel blockade on contractile responses*

To assess whether alterations in K<sup>+</sup> channel activity are involved in adrenergic nerve-mediated contraction of pulmonary artery, we determined the contractile responses to EFS before and after the addition of various K<sup>+</sup> channel blockers including iberiotoxin ( $10^{-8}$  M) (Peptide Institute Inc., Osaka, Japan), a big conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel blocker [8], glibenclamide ( $10^{-5}$  M), an ATP-sensitive K<sup>+</sup> channel blocker [9], and apamin ( $10^{-8}$  M) (Peptide Institute Inc.), a small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel blocker [10]. The concentration of each blocker was chosen based on the previous reports [6, 7]. We first obtained the baseline responses to EFS at increasing frequencies of stimulation (1–50 Hz); we then added each K<sup>+</sup> channel blocker to the chamber, and after 15 min we repeated the measurements. To characterize the frequency-response curves, the electric stimulus frequency required to produce a half maximal contraction (ES50) was determined by linear regression analysis.

Because only iberiotoxin and apamin increased the EFS-induced contraction, we used these two blockers in the following experiments. To study the concentration-dependent effects of Ca<sup>2+</sup>-activated K<sup>+</sup> channel blockers, after establishing the contractile responses to EFS at 10 Hz, various concentrations of iberiotoxin or apamin ( $10^{-9}$ – $10^{-7}$  M) were cumulatively added, while the measurements were repeated 15 min after the application of each concentration.

To determine whether the site of action of K<sup>+</sup> channel blockers was prejunctional or postjunctional in the adrenergic neural pathway, we examined the effect on noradrenaline-induced contraction. To do so, noradrenaline ( $10^{-8}$ – $10^{-4}$  M) (Sigma Chemical Co., St Louis, MO, USA) was cumulatively added in half-molar increments at 5 min intervals or 2 min after the stable plateau was achieved, whichever was the longer period, while the contractile responses to each concentration was determined. The tissues were then washed with KH solution, each K<sup>+</sup> channel blocker was added, and 15 min later a second concentration-response curve was generated. Our preliminary experiments showed that the first and second contractile responses were similar with the addition of KH solution alone in the experiments with EFS and noradrenaline. To characterize the concentration-response curves, the negative logarithm of the molar concentration of noradrenaline necessary to produce a half-maximal contraction (pD<sub>2</sub>) was determined by linear regression analysis.

#### *Measurement of noradrenaline release*

To confirm whether K<sup>+</sup> channels are actually involved in adrenergic neurotransmission, we measured the release of noradrenaline from adrenergic nerves. Pulmonary arte-

rial rings were incubated for 60 min at 37°C in KH solution containing 0.5  $\mu$ M <sup>3</sup>H-noradrenaline (specific activity 43.9 Ci·mmol<sup>-1</sup>; Amersham, Tokyo, Japan). After rinsing, the tissues were mounted in organ chambers and perfused at a rate of 1 mL·min<sup>-1</sup> for 60 min with fresh KH solution. The preincubated rings were then stimulated four times with EFS at 10 Hz for 3 min with 20 min intervals. The superfusate was collected at the end of each stimulation in a 3 mL aliquot, and was called S1, S2, S3 and S4. Iberiotoxin ( $10^{-8}$  M), apamin ( $10^{-8}$  M), or glibenclamide ( $10^{-5}$  M) was added 15 min before S4 to the superfusing solution. The EFS-evoked overflow of total tritium was calculated by subtraction of basal overflow, and the drug effect was expressed as the ratio between the overflow evoked by S4 and that evoked by S3.

#### *Statistics*

All data are expressed as means  $\pm$  SEM. Statistical analysis was performed by analysis of variance (ANOVA) followed by either Turkey's test for multiple comparisons or by Student's t-test. A p-value of less than 0.05 was considered statistically significant.

## **Results**

#### *Contractile responses*

Addition of K<sup>+</sup> channel blockers to the organ chambers at concentrations used in the present experiments did not change the resting tension of canine pulmonary arterial rings. As shown in figure 1, incubation of tissues with iberiotoxin ( $10^{-8}$  M) potentiated the contractile responses to EFS at stimulus frequencies of 1–30 Hz, so that the ES50 value was decreased from  $18.2 \pm 3.5$  to  $7.4 \pm 2.3$  Hz ( $p < 0.01$ ;  $n = 12$ ). Apamin ( $10^{-8}$  M) likewise produced a leftward displacement of the frequency-response curves for EFS, causing a decrease in the ES50 value from  $16.8 \pm 2.2$  to  $11.4 \pm 2.0$  Hz ( $p < 0.05$ ;  $n = 10$ ). By contrast, glibenclamide had no effect on the contractile responses to EFS (table 1). The EFS (10 Hz)-induced contraction was augmented by iberiotoxin and apamin in a concentration-dependent manner, with the maximal increase from the baseline response being  $46.3 \pm 5.2\%$  ( $p < 0.001$ ;  $n = 8$ ) and  $22.0 \pm 3.8\%$  ( $p < 0.01$ ;  $n = 8$ ), respectively (fig. 2).

To determine the site of action of iberiotoxin and apamin in the adrenergic neural pathway, we examined the effects of these blockers on the contractile responses to exogenously applied noradrenaline. In contrast to the effect on the responses to EFS, iberiotoxin and apamin did not significantly alter the contractile responses to noradrenaline (table 1 and fig. 1).

#### *Noradrenaline release*

In pulmonary arterial rings incubated for 60 min in KH solution containing 0.5  $\mu$ M <sup>3</sup>H-noradrenaline and superfused for 60 min with fresh KH solution, the <sup>3</sup>H-overflow evoked by EFS for 3 min was stabilized. Addition of K<sup>+</sup> channel blockers to the superfusing solution did not alter the baseline <sup>3</sup>H-overflow. However, iberiotoxin and apamin but not glibenclamide increased the EFS-evoked <sup>3</sup>H-overflow ( $p < 0.001$  and  $p < 0.01$ , respectively,  $n = 10$ ) (fig. 3).

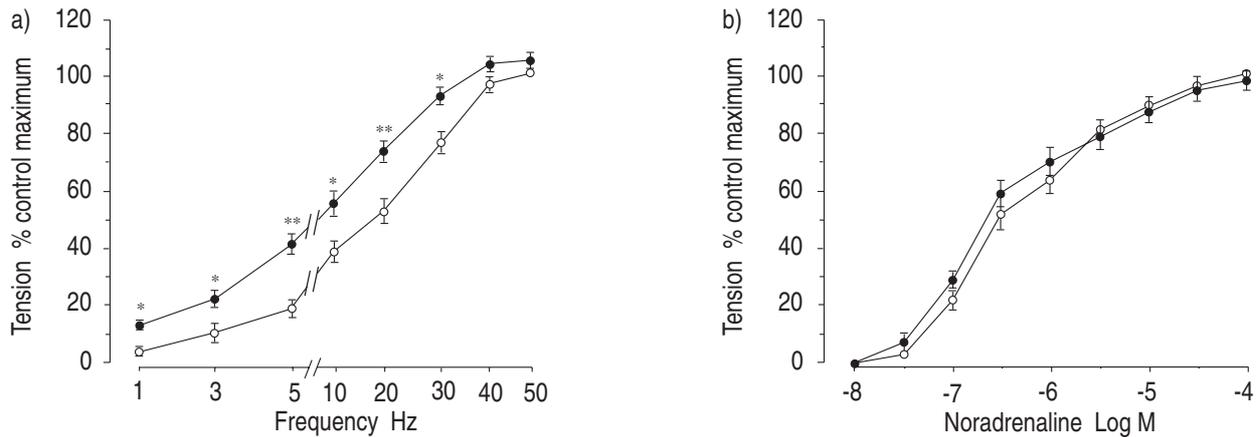


Fig. 1. – Effect of iberiotoxin on contractile responses of canine pulmonary artery to a) electrical field stimulation and b) noradrenaline. After determining baseline responses (—○—), tissues were washed, iberiotoxin ( $10^{-8}$  M) was added, and the measurements were repeated (—●—). Each point represents the mean  $\pm$  SEM ( $n=12$ ). Values are presented as percentage of maximal baseline responses. \*, \*\*:  $p<0.05$ ,  $p<0.01$ , versus baseline responses.

Table 1. – Effects of K<sup>+</sup> channel blockers on contractile responses of canine pulmonary artery

	EFS ES50 Hz		Noradrenaline pD <sub>2</sub>	
	Baseline	Blocker	Baseline	Blocker
Iberiotoxin	18.2 $\pm$ 3.5	7.4 $\pm$ 2.3**	6.5 $\pm$ 0.3	6.6 $\pm$ 0.3
Apamin	16.8 $\pm$ 2.2	11.4 $\pm$ 2.0*	6.5 $\pm$ 0.4	6.8 $\pm$ 0.3
Glibenclamide	18.3 $\pm$ 2.6	17.7 $\pm$ 2.5	6.2 $\pm$ 0.3	6.4 $\pm$ 0.4

EFS: electrical field stimulation; ES50: stimulus frequency required to produce a half-maximal baseline response; pD<sub>2</sub>: negative logarithm of molar concentration of noradrenaline required to produce a half-maximal baseline response. Data are expressed as mean  $\pm$  SEM ( $n=9-12$ ). \*, \*\*, \*\*\*:  $p<0.05$ ,  $p<0.01$ , significantly different from corresponding baseline values.

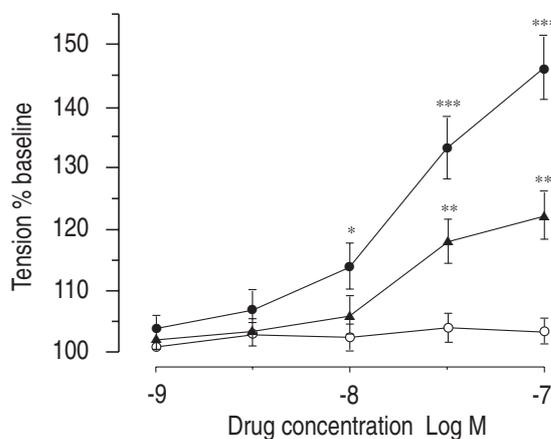


Fig. 2. – Concentration-dependent effects of iberiotoxin (—●—) and apamin (—▲—) on contractile responses of canine pulmonary artery to electrical field stimulation at 10 Hz. Each point represents the mean  $\pm$  SEM ( $n=8$ ). Values are presented as percentage of the baseline responses obtained before drug addition. \*, \*\*, \*\*\*:  $p<0.05$ ,  $p<0.01$ ,  $p<0.001$  versus response to solvent alone (—○—).

## Discussion

Our *in vitro* studies demonstrate that large and small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels may prejunctionally inhibit adrenergic nerve-mediated contraction of isolated canine pulmonary artery. We found that the addition of iberiotoxin, a big conductance Ca<sup>2+</sup>-activated K<sup>+</sup> chan-

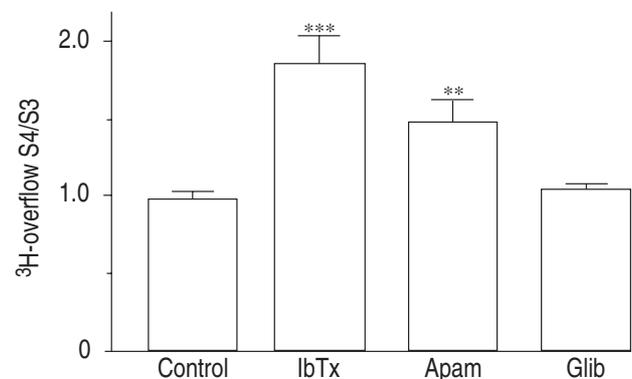


Fig. 3. – Effect of K<sup>+</sup> channel blockers on electrically-evoked <sup>3</sup>H-overflow from canine pulmonary artery pre-incubated with <sup>3</sup>H-noradrenaline. Four periods of electrical field stimulation (10 Hz) were applied with 20 min intervals (S1–S4). Iberiotoxin (IbTx,  $10^{-8}$  M), apamin (Apam,  $10^{-8}$  M), glibenclamide (Glib,  $10^{-5}$  M), or the solvent (Control) was added 15 min before S4. Data are presented as mean  $\pm$  SEM; ( $n=10$ ). Values are presented as ratio of <sup>3</sup>H-overflow between S4 and S3. \*\*, \*\*\*:  $p<0.01$ ,  $p<0.001$  versus control values.

nel blocker [8], and apamin, a small conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel blocker [10], at concentrations up to  $10^{-7}$  M, potentiated the contractile responses to EFS but did not alter those to exogenously applied noradrenaline. These findings indicate that the effects of iberiotoxin and apamin were not associated with concomitant alterations of vascular smooth muscle functions, such as enhanced adrenergic receptor binding, decreased uptake of noradrenaline by tissues, or inhibition of noradrenaline metabolism. Therefore, iberiotoxin and apamin are probably acting

on prejunctional K<sup>+</sup> channels in the adrenergic nerve fibres, and the augmentation of the EFS-induced contraction might be attributable to the increase in the exocytotic release of noradrenaline. However, Ca<sup>2+</sup>-activated K<sup>+</sup> channels are present on vascular smooth muscle cells, and our preliminary experiment showed that a higher concentration of iberiotoxin ( $10^{-6}$  M) by itself increased the baseline tension of pulmonary artery segments. The reason for the lack of effect with lower concentrations of iberiotoxin on the noradrenaline-induced contraction is uncertain, but this could be due to the difference in the sensitivity of K<sup>+</sup> chan-

nels between nerve cells and smooth muscle cells. Similar findings have previously been reported [6, 7].

In contrast to the effects of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channel blockers, addition of glibenclamide at a concentration sufficient to block ATP-sensitive  $\text{K}^+$  channels [9] had no effect on the contractile responses to EFS or noradrenaline, suggesting that ATP-sensitive  $\text{K}^+$  channels may not be involved in the regulation of adrenergic neurotransmission. However, these results do not exclude a role for ATP-sensitive  $\text{K}^+$  channels patch clamp studies by CLAPP and GURNEY [11] demonstrated that these channels were pre-sent on rabbit pulmonary arterial smooth muscle cells. Our results are in accordance with a recent report [12] showing that nonselective  $\text{K}^+$  channel blockers, such as tetra-ethylammonium and 4-aminopyridine, increased pulmonary vascular tone in the perfused rat lungs, whereas glibenclamide was without effect.

To further assess the involvement of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels and ATP-sensitive  $\text{K}^+$  channels in adrenergic neurotransmission, we directly measured the release of tritium from pulmonary artery preincubated with  $^3\text{H}$ -noradrenaline. Addition of iberiotoxin and apamin had no effect on the baseline release of  $^3\text{H}$ -overflow but significantly increased the EFS-evoked  $^3\text{H}$ -overflow. Glibenclamide, in contrast, did not affect the  $^3\text{H}$ -overflow evoked by EFS. These findings are compatible with the results of contraction studies, and confirm that big conductance and small conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels but not ATP-sensitive  $\text{K}^+$  channels play a regulatory role in the exocytotic release of noradrenaline from adrenergic nerves in the pulmonary artery.

It has been shown that several types of  $\text{K}^+$  channels, including voltage sensitive  $\text{K}^+$  channels [13], big conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels [14] and small conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels [15], are present on nerve cells. When EFS is applied, depolarization of nerve fibres activates voltage-gated  $\text{Ca}^{2+}$  channels, through which the entry of  $\text{Ca}^{2+}$  occurs to effect the release of neurotransmitters [16]. The rise in intracellular  $\text{Ca}^{2+}$  concentration may open  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels, which in turn causes efflux of  $\text{K}^+$ , thereby leading to membrane hyperpolarization. This hyperpolarization inhibits  $\text{Ca}^{2+}$  influx through voltage-gated  $\text{Ca}^{2+}$  channels and increases  $\text{Na}^+$ - $\text{Ca}^{2+}$  exchange [17]. In addition, activation of presynaptic  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels could inhibit neurotransmission by shortening the action potential and reducing  $\text{Ca}^{2+}$  influx [18]. Consequently, these processes in the adrenergic nerve fibres inhibit the release of noradrenaline. Thus, both big conductance and small conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels appear to be functioning as negative feedback mechanisms by counteracting  $\text{Ca}^{2+}$ -associated facilitation of adrenergic neurotransmission in the pulmonary artery.

In conclusion, our present studies provide further evidence that  $\text{K}^+$  channels play a role in the regulation of adrenergic neurotransmission. Activation of big conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels and small conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels inhibits the exocytotic release of noradrenaline and, hence, reduces the adrenergic component of pulmonary vasoconstriction.

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$^3\text{H}$ -overflow.

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