Selegiline, an MAO-B inhibitor, attenuates airway smooth muscle contraction in the rat trachea

Maki Yoshimura, Osamu Shibata, Masataka Saito, Masakazu Yamaguchi, Kenji Nishioka, Tetsuji Makita and Koji Sumikawa

Abstract

Selegiline is widely used for Parkinson's disease and sometimes for Alzheimer's disease. It is reported to affect intracellular Ca^{2+} concentration. Since intracellular Ca^{2+} is partly regulated by phosphatidylinositol (PI) response and is important for smooth muscle contraction, selegiline may affect airway smooth muscle tension. We examined the effects of selegiline on acetylcholine (ACh)- and KCl-induced contractile and PI responses in rat trachea. The trachea was cut into 3-mm-wide ring segments or 1-mm-wide slices. ACh ($3\,\mu M$, 50% effective dose) or KCl ($40\,mM$) was added, and ring relaxation was induced by the addition of selegiline. Tracheal slices were incubated with [^{3}H]myo-inositol and $3\,\mu M$ ACh in the presence of selegiline, and [^{3}H]inositol monophosphate (IP_1) was measured. Selegiline dose-dependently attenuated ACh- and KCl-induced tracheal ring contractions. Fifty-percent inhibitory doses (ID50) of selegiline against ACh- and KCl-induced contraction were $120 \pm 30\,\mu M$ and $80 \pm 20\,\mu M$, respectively. Basal and ACh-induced IP_1 accumulation were $2.20 \pm 0.20\,Bq$ and $7.88 \pm 0.23\,Bq$, respectively, and selegiline at a dose of $1000\,\mu M$ attenuated ACh-induced IP_1 accumulation ($5.44 \pm 0.30\,Bq$). These results suggest that selegiline inhibits contractile responses through the inhibition of voltage-operated Ca^{2+} channels and the PI response.

Introduction

Selegiline, a monoamine oxidase (MAO)-B inhibitor, is widely used for Parkinson's disease and sometimes for Alzheimer's disease. Ebadi et al (2002) reported that selegiline induced neuroprotection in patients with Parkinson's disease and in addition to MAO-B inhibitory action, it had various other actions. They also reviewed that pre-treatment with selegiline could protect neurons against neurotoxins, such as 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). Kass et al (1988) found that MPTP caused rapid depletion of mitochondrial Ca^{2+}, which was followed by a marked and sustained elevation of cytosolic Ca^{2+}, and concluded that Ca^{2+} was of major importance in the mechanism of MPTP-induced toxicity in hepatocytes. Since selegiline protects neurons, it may decrease cytosolic Ca^{2+} concentration. On the other hand, Itoh et al (1998) examined the effects of selegiline on the intracellular Ca^{2+} concentration of primary cultures of rat striatal and mesencephalic neuronal cells by the use of a Ca^{2+}-imaging analyser, and found that selegiline at $1\,\mu M$ and $10\,\mu M$ induced a transient inflow of extracellular Ca^{2+} through voltage-dependent N-type Ca^{2+} channels. They also found that all cells displayed an increase in intracellular Ca^{2+} concentration. It is not clear whether selegiline increased or decreased intracellular Ca^{2+} concentration. Since intracellular Ca^{2+} homeostasis is partly regulated by phosphatidylinositol (PI) response (Berridge 1983), selegiline may affect the PI response.

The number of patients treated with selegiline for the treatment of Parkinson's disease and Alzheimer's disease is increasing due to the increase in the rate of these diseases as the population ages. Since bronchial asthma, or hyper-responsiveness, is prevalent in the elderly population, this condition might become a serious risk for any patients undergoing anaesthesia before surgery. It seems probable that selegiline may affect airway smooth muscle tension through effects on the intracellular Ca^{2+} concentration. Thus, we

Department of Anesthesiology, Nagasaki University School of Medicine, Nagasaki 852-8501, Japan

Maki Yoshimura, Osamu Shibata, Masataka Saito, Masakazu Yamaguchi, Kenji Nishioka, Tetsuji Makita, Koji Sumikawa

Correspondence: M. Yoshimura, Department of Anesthesiology, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. E-mail: makichan11@hotmail.com

examined the effects of selegiline on acetylcholine (ACh)- and KCl-induced contractile and PI responses of rat trachea.

Materials and Methods

Drugs

Selegiline was donated by Fujimoto Pharmaceutical Corp. (Osaka, Japan). Acetylcholine, propranolol, yohimbine and glibenclamide were purchased from Sigma (St Louis, MO) and [^{3}H]*myo*-inositol was purchased from Amersham (Tokyo, Japan).

Animals

The studies were conducted under guidelines approved by the Animal Care Committee of Nagasaki University School of Medicine. Thirty-eight male Wistar rats (Charles River, Yokohama, Japan), 250–350 g, were used for the experiments. The rats were anaesthetized with pentobarbital (50 mg kg^{-1}, i.p.), and their tracheas were rapidly isolated.

Contractile response

Each trachea was cut into 3-mm-wide ring segments with a McIlwain tissue chopper (The Mickle Laboratory Engineering, Gomshall, UK). Each tracheal ring segment was suspended between two stainless-steel hooks and placed in a 5-mL water-jacketed organ chamber (Kishimotoika, Kyoto, Japan) containing Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4.7, CaCl$_2$ 1.3, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 25, glucose 11, Na$_2$-EDTA 0.05). The solution was continuously aerated with 95% O$_2$–5% CO$_2$ at 37°C. Isometric tension was measured using an isometric transducer (Kishimotoika, Kyoto, Japan) and change in isometric force was recorded using a MacLab system (Milford, MA). The resting tension was adjusted periodically to 1.0 g during the equilibration period. The ring was washed every 15 min and re-equilibrated to baseline tension for 60 min (Time 0).

We determined the ED50 of ACh, the concentration providing 50% of maximal contraction of the rat tracheal ring. The ring contraction was induced by stepwise cumulative additions of ACh, 0–1000 μM in final concentration, and the ED50 was determined.

To examine the effects of α_2 and β-adrenoceptors on the relaxation by selegiline of ACh-induced contraction, we used yohimbine, an α_2-adrenoceptor antagonist, and propranolol, a β-adrenoceptor antagonist. At time 0, yohimbine or propranolol (10 μM) was added. After 15 min, ACh (3 μM) was added and after a further 10 min, ring tension was examined by cumulative additions of selegiline, in final concentrations of 0–1000 μM.

To examine the role of the ATP-sensitive potassium (K$_{ATP}$) channels in the relaxation by selegiline of ACh-induced contraction, we used glibenclamide, a K$_{ATP}$-channel blocker. At time 0, glibenclamide (10 μM) was added. After 15 min, ACh (3 μM in final concentration) was added and after a further 10 min, ring relaxation was induced by cumulative additions of selegiline in final concentrations of 0–1000 μM.

To examine the effects of selegiline on voltage-operated Ca^{2+} channels, we used KCl (40 mM in final concentration). At time 0, KCl was added and 30 min later, ring relaxation was induced by stepwise cumulative additions of selegiline in final concentrations of 0–1000 μM.

PI response

The technique of Brown et al (1984) was used. Inositol 1,4,5-trisphosphate (IP$_3$) is rapidly degraded into IP$_1$ and this is recycled back to PI via free inositol. Lithium inhibits the conversion of IP$_1$ to inositol. Thus, in the presence of Li$^+$, the accumulation rate of IP$_1$ reflects the extent of PI response. We measured [^{3}H]IP$_1$ in tracheal slices incubated with [^{3}H]*myo*-inositol. The trachea was cut longitudinally and chopped into 1-mm-wide pieces with a McIlwain tissue chopper. Three pieces of the tracheal slices were placed in small flat-bottomed tubes and pre-incubated for 15 min in Krebs-Henseleit solution containing 5 mM LiCl. The solution was continuously aerated with 95% O$_2$–5% CO$_2$. A sample of 0.5 μCi [^{3}H]*myo*-inositol was then added to each tube (final concentration 0.1 μM in a 300-μL incubation volume). All of the tubes were subsequently flushed with 95% O$_2$–5% CO$_2$, capped, set in a shaking bath at 37°C and incubated for 30 min (time 0).

We also examined the effects of selegiline on ACh-induced IP$_1$ accumulation in rat tracheal slices. At time 0, varying doses (0, 1, 10, 100 and 1000 μM) of selegiline were added to the tracheal-slice suspensions and the tubes were flushed with 95% O$_2$–5% CO$_2$. After a period of 15 min, ACh (3 μM in final concentration) or none was added. After an additional 60 min, the reaction was stopped with 940 μL chloroform–methanol (1:2 v/v). Chloroform and water were then added (300 μL each) and the phases were separated by centrifugation at 90 g over a period of 5 min.

The [^{3}H]IP$_1$ was separated from [^{3}H]*myo*-inositol in the 750 μL water phase by column chromatography, using Dowex AG 1-X8 resin (Bio Rad, Richmond, CA) in the formate form. The [^{3}H]IP$_1$ formed in the tracheal slices was counted with a liquid scintillation counter and the counts were measured in becquerels (Bq). The counts of the blank samples (no slices present) were subtracted from all of the other counts to obtain the experimental data.

Statistical analysis

Data were expressed as mean $\pm$ s.e. The results were subjected to one-way or two-way analysis of variance. Post-hoc analysis was then performed using Scheffe's F-test. A P value < 0.05 was considered significant.

Results

Figure 1 shows the effects of acetylcholine (ACh) on resting tension of rat trachea. The ED50 of ACh was 3.0 $\pm$ 0.9 μM. Figure 2 shows a typical recording of the effects of selegiline on ACh-induced contraction of rat tracheal rings. The
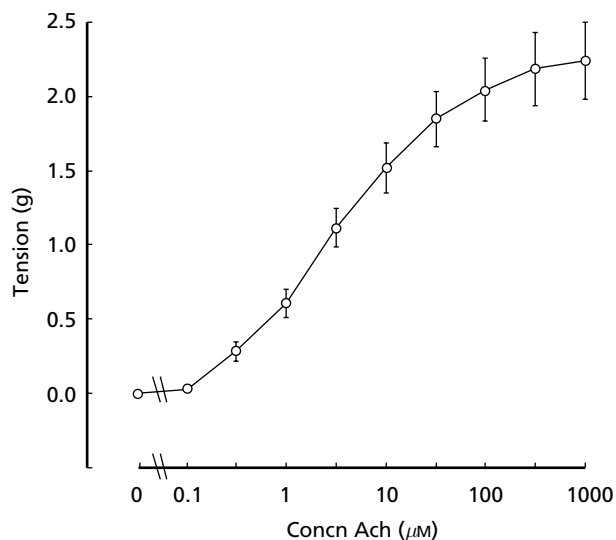
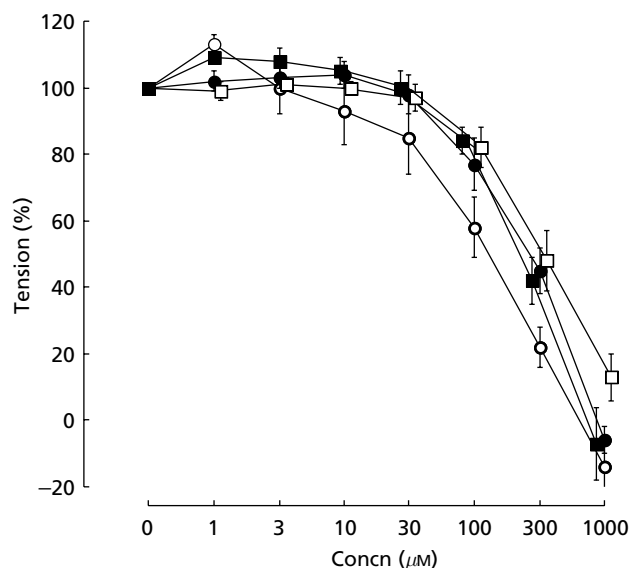**Figure 1** The effect of acetylcholine (ACh) on resting tension of rat trachea (mean $\pm$ s.e.m., n $=8$).
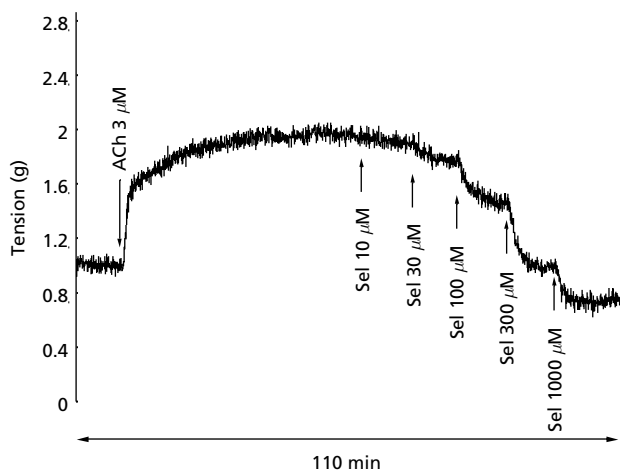
Figure 2 A typical recording of the effects of selegiline (Sel) on acetylcholine (ACh)-induced contraction of rat tracheal rings.

Figure 3 The effect of selegiline on the tracheal ring contraction induced by 3 μM acetylcholine in the absence (O) or presence of 10 μM propranolol (●), 10 μM yohimbine (□) or 10 μM glibenclamide (■) (mean $\pm$ s.e.m., n $=7$ or 8).

Figure 4 The effect of selegiline on contraction of rat tracheal rings induced by 40 mM KCl (mean $\pm$ s.e.m., n $=7$).
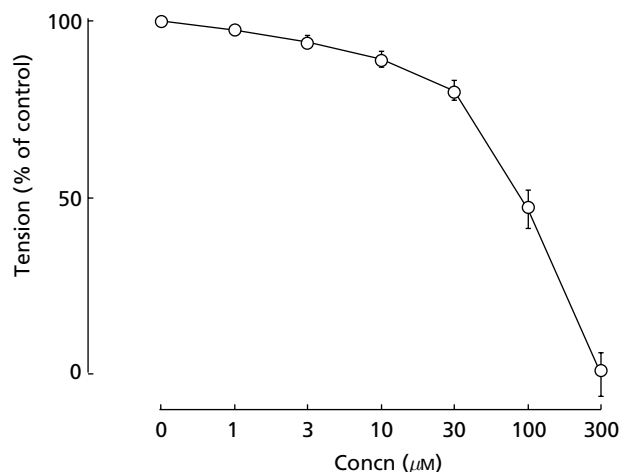
IC50 of selegiline on ACh-induced tracheal ring contraction in the absence or presence of propranolol, yohimbine or glibenclamide was $85 \pm 25 \mu$M, $250 \pm 60 \mu$M, $280 \pm 70 \mu$M and $230 \pm 50 \mu$M, respectively (Figure 3). Selegiline attenuated KCl-induced tracheal ring contraction (Figure 4). Figure 5 shows the effects of selegiline on basal and ACh- and KCl-induced IP$_1$ accumulation of rat tracheal slices. ACh significantly stimulated IP$_1$ accumulation ($P < 0.001$) but KCl had no effect. Selegiline attenuated ACh-induced IP$_1$ accumulation at a dose of 1000 μM ($P < 0.01$).

Discussion

In this study, we found that selegiline attenuated the ACh-induced contractile and PI responses of rat trachea. We

also found that selegiline attenuated KCl-induced contraction. The action of selegiline on airway smooth muscle may therefore involve one of the following mechanisms.

The relaxant effect of selegiline may be mediated via inhibition of voltage-operated Ca^{2+} channels. Intracellular Ca^{2+} is mainly regulated by the influx of extracellular Ca^{2+} through membrane-associated Ca^{2+} channels and Ca^{2+} release from intracellular stores. The former is regulated by membrane potential and the latter is regulated by cytosolic Ca^{2+} and inositol 1,4,5-trisphosphate (IP$_3$). Itoh et al (1998) observed the effects of selegiline on intracellular Ca^{2+} in cultured neuronal cells and found a transient
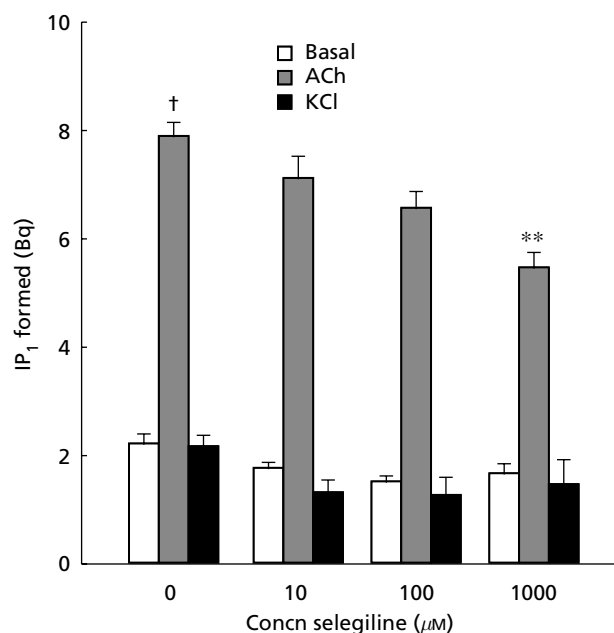
Figure 5 The effect of selegiline on basal inositol monophosphate (IP$_1$) accumulation of rat tracheal slices and IP$_1$ accumulation induced by 3 μM acetylcholine (ACh) or 40 mM KCl (mean $\pm$ s.e.m., n = 8–10). $^{\dagger}P < 0.001$ vs basal; $**P < 0.01$ vs 0 μM selegiline.

intracellular influx of Ca^{2+}. On the other hand, Kass et al (1988) reported that pargyline, an MAO-B inhibitor, prevented a rise in cytosolic Ca^{2+} in isolated hepatocytes. In this study, higher doses of selegiline attenuated KCl-induced contraction. Thus, selegiline would inhibit voltage-operated Ca^{2+} channels, resulting in attenuation of rat tracheal rings.

Selegiline may act directly on muscarinic receptors of airway smooth muscle. We examined the effects of selegiline on ACh-induced contractile properties and PI responses, and found that ACh-induced contractile and PI responses were inhibited by selegiline dose dependently. Muscarinic receptor agonists activate PI response through G-protein-coupled phospholipase C (PLC) and subsequently increases Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} influx from extracellular fluid. Thus, selegiline would inhibit the activation of G-protein-coupled PLC in PI response in airway smooth muscle cell membrane.

Selegiline, which decrease Ca^{2+}-channel opening, may decrease Ca^{2+}-stimulated PLC activity and subsequently decrease the rate of ACh-induced PI response because the PI response can also be activated by an increase in intracellular Ca^{2+}. KCl opens the voltage-operated Ca^{2+} channels, resulting in an increase in intracellular Ca^{2+}. However, since in this study, KCl did not stimulate the PI response and selegiline did not affect KCl-induced PI response, it is unlikely that selegiline would inhibit PI response through the inhibition of voltage-operated Ca^{2+} channels.

The relaxant effects of selegiline may be mediated via airway adrenoceptors. Torok et al (1987) reported that a high concentration of selegiline potentiated nerve-stimulation-evoked release of noradrenaline (norepinephrine) from the isolated main pulmonary artery of the rabbit. ThyagaRajan & Quadri (1999) observed that selegiline stimulated the release of catecholamines in the rat medial basal hypothalamus. Knoll et al (1996) reported that selegiline acted primarily as a potent stimulant of action potential transmitter release coupling in the catecholaminergic neurons. Thus, in addition to the inhibition of metabolic degradation of catecholamines, stimulation of the release of noradrenaline from catecholaminergic nerve terminals in the airway smooth muscle may increase noradrenaline to activate β-adrenoceptors and α_2-adrenoceptors. Shibata et al (2000) reported that clonidine, an α_2-adrenoceptor agonist, attenuated the carbachol-induced contractile and PI responses of rat trachea and Kamikawa & Shimo (1990) reported that the inhibitory effect of noradrenaline on electrical-field-stimulation-induced contraction of guinea-pig bronchial rings was prevented with both propranolol and yohimbine. However, in this study, propranolol and yohimbine did not alter the relaxant effects of selegiline. Thus, the relaxant effect of selegiline on ACh-induced contraction of rat tracheal rings would not be mediated via adrenoceptors.

The relaxant effects of selegiline may be mediated via activation of K$_{ATP}$ channels. K$_{ATP}$ channels exist in cell membranes such as smooth muscle and neurons. K$_{ATP}$-channel openers have a wide range of effects and are theoretically useful in patients with asthma. When K$_{ATP}$ channels are opened in airway smooth muscle cell membranes, the increase in K$^+$ efflux shifts the membrane potential in a hyperpolarizing direction towards the K$^+$ equilibrium potential. Hyperpolarization prevents Ca^{2+} entry through voltage-operated Ca^{2+} channels, resulting in smooth muscle relaxation in the airway (Quast 1993). However, in our study, glibenclamide, a K$_{ATP}$-channel blocker, could not abolish the attenuation caused by selegiline on ACh-induced tracheal smooth muscle contraction. Thus, selegiline could not cause activation of ATP-sensitive K$^+$ channels of rat tracheal smooth muscle.

In this study, the effective concentration of selegiline on the ACh-induced contraction of rat tracheal rings was 100 μM ($P < 0.05$). The amount of orally administered selegiline reaching the systemic circulation is less that 10% of the total. Following an oral dose of 10 mg of selegiline, the mean peak plasma concentration is approximately 2 μg L^{-1}, about 9 nM (Mahmood 1997). Although the concentration required for tracheal smooth muscle relaxation in this study is not clinically relevant, the results suggest a potential benefit of the drug in patients with asthma.

Conclusion

Selegiline inhibits ACh- and KCl-induced contractile responses and it attenuates ACh-induced IP$_1$ accumulation. These results suggest that selegiline inhibits the contractile responses of rat trachea mainly through the inhibition of voltage-operated Ca^{2+} channels, and partly through the PI response.

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