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Effects of the K^+ channel blockers paspalitrem-C and paxilline on mammalian smooth muscle

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Abstract

The tremorgenic alkaloids, paxilline and paspalitrem-C (0.1–10 μM), increased the spontaneous contractility of guinea-pig and rat urinary bladder, and rat duodenum, and induced tension in guinea-pig trachea. These effects are ascribed to blockade of high-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channels. Paxilline potentiated the charybdotoxin-induced stimulation of guinea-pig detrusor muscle; this is consistent with the alkaloid's ability to allosterically enhance the binding of charybdotoxin to smooth muscle membranes (Knaus et al., 1994). Paspalitrem-C and paxilline did not affect the myogenic activity of isolated portal vein from guinea-pig, which is insensitive to charybdotoxin, or of that from rat which is stimulated by charybdotoxin. Paxilline and paspalitrem-C also differed from charybdotoxin in that the alkaloids did not consistently elicit tension in guinea-pig aortic rings. These discrepancies are attributed to differences in relative potency, sites and/or mechanisms of action of the indole alkaloids vs. peptidyl blockers of the BK_{Ca} channel.

Keywords: Tremorgenic indole alkaloid; Ca^{2+}-activated K^+ channel; Charybdotoxin; Iberiotoxin; Excitation-contraction coupling

1. Introduction

High-conductance, Ca^{2+}-activated K^+ channels, also known as BK_{Ca} channels, are present in several smooth muscle cells, where they modulate excitation-contraction coupling processes (Winquist et al., 1989; Suarez-Kurtz et al., 1991; Jones et al., 1990, 1993; Huang et al., 1993; Nelson et al., 1995). Thus, BK_{Ca} channels have been implicated in setting the resting membrane potential and controlling Ca^{2+} influx via voltage-dependent channels. Additionally, in some cell types, BK_{Ca} channels play a role in the repolarization phase of action potentials and are capable of generating spontaneous transient outward currents that oppose membrane depolarization (Rudy, 1988; Carl and Sanders, 1989; Saunders and Farley, 1991). BK_{Ca} channels of mammaliam smooth muscle are potently blocked by nanomolar concentrations of charybdotoxin and iberiotoxin, two peptide toxins extracted from venom of the scorpions *Leiurus quinquestriatus* (Miller et al., 1985; Gimenez-Gallego et al., 1988) and *Buthus tamulus* (Galvez et al., 1990), respectively. Patch-clamp recordings of unitary BK_{Ca} channel currents revealed that blockade by charybdotoxin and iberiotoxin is characterized by long silent periods alternating with normal channel activity, due to the fast binding and slow dissociation kinetics of the peptides in the pore of the channel (Anderson et al., 1988; Giangiacomo et al., 1992). Because of their selectivity and potency, charybdotoxin and iberiotoxin are valuable experimental tools for exploring the functional role of BK_{Ca} channels in excitation-contraction coupling in smooth muscle (Suarez-Kurtz et al., 1991).

A recent study from one of our laboratories (Knaus et al., 1994) revealed that tremorgenic indole alkaloids obtained from fungi belonging to the genera *Penicillium*, *Aspergillus* and *Claviceps* are apparently the most potent and selective non-peptidyl inhibitors of the BK_{Ca} channel identified to date. This observation prompted us to study the effects of two of these fungal mycotoxins on smooth muscle contractility; viz. paspalitrem-C and paxilline produced by the fungi *Phomopsis* sp. and *Penicillium paxilline*, respectively. These tremorgenic alkaloids are repre-

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sentative members of a diverse group of compounds (Cole and Cox, 1981) which bind to a site on the BK_{Ca} channel that is allosterically coupled to the charybdotoxin receptor in the pore of the channel. Some of these agents have opposite effects on binding of [^{125}I]charybdotoxin to the channel: paspalitrem-C reduces, whereas paxilline enhances toxin binding. Nevertheless, both alkaloids markedly inhibit BK_{Ca} channels in single channel records from excised membrane patches of smooth muscle cells, with greatest potency when they are added to the intracellular face of the channel (Knaus et al., 1994).

2. Materials and methods

2.1. Preparations

Experiments were performed at 37°C on tissues obtained from adult guinea-pigs or Wistar rats after death from ether inhalation. Guinea-pig provided portal vein and urinary bladder strips, thoracic aorta and tracheal rings. Rats provided portal vein, duodenum and bladder strips. For recording muscle tension, the preparations were mounted between two metal stirrups, the lower of which was fixed and the upper attached to a rigid wire connected to a force-displacement transducer (Grass FT-03; Grass Instruments, Quincy, MA, USA). The transducer signals were amplified and recorded on a Grass polygraph (Model 7). The amplified signals from detrusor muscle, duodenum and portal vein, preparations which exhibited spontaneous motility, were fed into an integrator (Grass 7P10) for quantitation of myogenic activity. A 1-g load was initially applied to all preparations, except guinea-pig aortic rings in which the load was 0.5 g.

2.2. Solutions

The physiological saline solution (PSS), a modified Krebs-Henseleit solution, had the following composition (in mmol): NaCl 120, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.1, $NaHCO_3$ 15, NaH_2PO_4 1.2, glucose 11, and Hepes 10. The pH of this solution after equilibration with 95% O_2 and 5% CO_2 was 7.3 at 37°C. The cyclooxygenase inhibitor, indomethacin (1.4 μM), was added to the PSS bathing the tracheal rings in order to reduce spontaneous tone development due to released prostanoids (Jones et al., 1990). Stock solutions of paspalitrem-C (obtained from a *Phomopsis* species of fungi, as previously described by Bills et al., 1992 and Knaus et al., 1994) and paxilline (Sigma, St. Louis, MO, USA) were prepared in dimethylsulfoxide (DMSO). These solutions were diluted appropriately to obtain the required concentration of agent in the muscle chamber. Because of the limited solubility of paspalitrem-C and paxilline in PSS, and the need to keep the final concentration of DMSO in the chamber below 0.1%, these drugs were tested at concentrations in the range of 0.1–10 μM. Synthetic charybdotoxin, which is identical in its biological activity to natural toxin (Sugg et al., 1990), was used in some experiments.

2.3. Experimental protocol

After an initial 60-min equilibration period in PSS, the preparations were exposed to increasing concentrations of either paspalitrem-C or paxilline. The effects of these compounds on muscle tension were quantified after 30-min exposure to each concentration. Integrated mechanical data from portal vein, bladder and duodenum were expressed relative to the basal integrated activity, recorded between 40 and 60 min after mounting the preparations in the muscle chamber, and immediately before exposure to the lowest concentration of the toxin being tested. Tension data from tracheal rings were expressed as percent of the maximum tension elicited by carbachol (10 μM). The data are plotted as means ± S.E.M. Student's *t*-test was used for statistical analysis of the data. The significance level was set at $P < 0.05$

3. Results

3.1. Effects of paspalitrem-C on contractility and tonus

Fig. 1 shows changes in the force of contraction and in the integrated mechanical activity of guinea-pig portal vein and urinary bladder after exposure to an indole alkaloid BK_{Ca} channel blocker. Paspalitrem-C (10 μM) increased the peak amplitude of the spontaneous contractions and the integrated myogenic activity of the bladder, but had no effect on portal vein. Data from four similar experiments indicated that 10 μM paspalitrem-C augmented the integrated activity of bladder 2.7 ± 0.3-fold above the basal

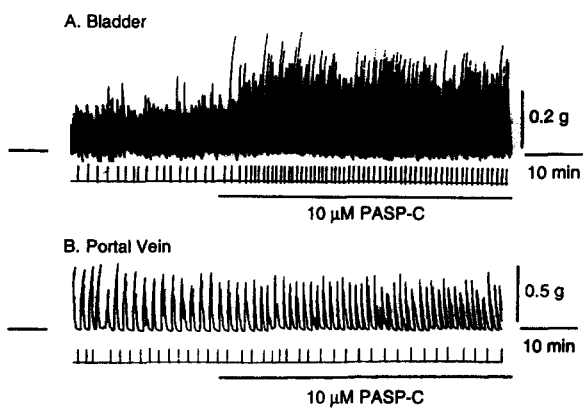

Fig. 1. Effect of paspalitrem-C on the contractility of guinea-pig smooth muscle. Isometric tension recordings were made from a strip preparation of urinary bladder and from portal vein before and during exposure to 10 mM paspalitrem-C. The integrated mechanical activity is shown below the tension recordings.

levels ($P < 0.05$), whereas 1 μM paspalitrem-C had no signficant effect on the integrated myogenic activity. Paspalitrem-C (0.1–10 μM) did not affect the resting tension of mechanically quiescent tracheal rings or aorta from guinea-pig (not shown).

3.2. Effects of paxilline on the spontaneous motility of smooth muscles

The effects of a structurally different indole alkaloid BK_{Ca} channel blocker, paxilline, on guinea-pig portal vein and urinary bladder were qualitatively similar to those described above for paspalitrem-C. Thus, paxilline (1–10 μM) did not affect the spontaneous motility of portal vein, but caused a dose-dependent stimulation of the contractility of urinary bladder strips (Fig. 2). The increase in the integrated myogenic activity induced by paxilline at 10 μM (9.6 $\pm$ 2.8-fold above the basal levels) was, on average, 3.6 times larger than that observed with 10 μM paspalitrem-C (see above).

Tissue selectivity was also observed in relation to the effects of paxilline on the spontaneous motility of rat smooth muscle preparations. This is illustrated in Fig. 3 with recordings from portal vein, duodenum and bladder strips from the same animal. In agreement with its effects on guinea-pig preparations, paxilline caused a dose-dependent increase in the spontaneous contractions and integrated myogenic activity of the bladder, but had no effect on portal vein. The contractility of the duodenum was also augmented by paxilline in a dose-dependent fashion. The magnitude of the paxilline-induced stimulation of the integrated myogenic activity of urinary bladder was quite variable among different strips. Preliminary observations (DeFarias, 1993) suggest that this variability may be related to the basal motility of the muscle strips. This question was not further explored in the present study.

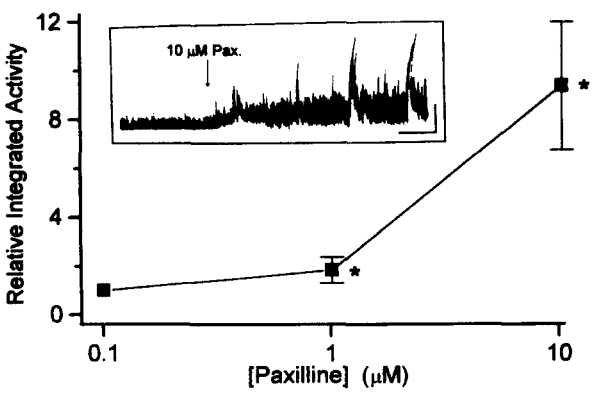

Fig. 2. Effect of paxilline on the integrated mechanical activity of guinea-pig urinary bladder strips. The data in the plot are expressed (mean $\pm$ S.E.M., $n = 5–11$) relative to basal activity. The inset shows an isometric tension recording from a urinary bladder strip before and during exposure to 10 mM paxilline. Calibration bars: horizontal, 5 min; vertical, 1 g.

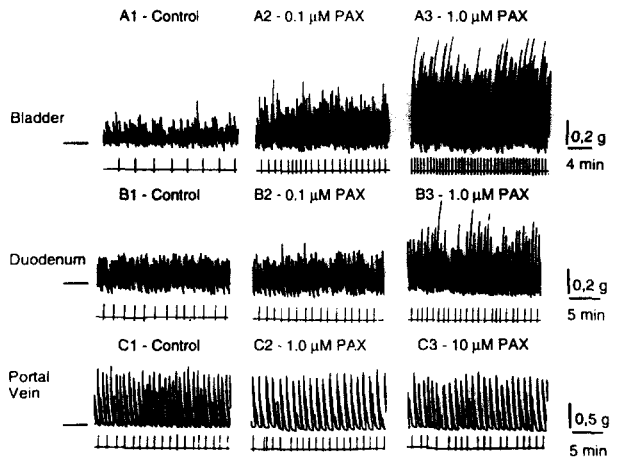

Fig. 3. Effect of paxilline on the contractility of rat smooth muscle. Isometric tension recordings were made from strip preparations of urinary bladder and duodenum, and from portal vein, before and between 30–50-min exposures to 0.1–10 mM paxilline. The integrated mechanical activity is shown below the tension recordings.

3.3. Effects of paxilline on guinea-pig aorta and tracheal rings

Paxilline (1 μM) had no effect on the tonus of mechanically quiescent aorta or tracheal rings. At 10 μM paxilline, tension was detected in 2 out of 6 aortas and in 7 out of 16 trachealis muscle preparations. The paxilline-induced tension in the 7 responsive tracheal rings developed slowly, reaching peak amplitudes of 23.4 $\pm$ 4.5% of the maximum (10 μM) carbachol-induced tension within 20 min. Tension oscillations were observed in 3 of these preparations during the exposure to paxilline (not shown). Five of the tracheal rings which did not respond to paxilline (10 μM), developed little (< 20% of the maximum carbachol-induced contraction) or no tension when challenged with 100 nM charybdotoxin.

3.4. Pharmacological interaction between paxilline and charybdotoxin

The observation (Knaus et al., 1994) that paxilline increases the binding of labeled charybdotoxin to the smooth muscle BK_{Ca} channel led us to explore the functional interaction of these two channel blockers with respect to smooth muscle motility. The experiments were peformed on guinea-pig bladder strips, which had displayed the highest sensitivity to paxilline in the present experiments. The data presented in Fig. 4 show that paxilline (1 μM) markedly potentiates the increase in integrated myogenic activity elicited by a submaximal concentration (20 nM) of charybdotoxin.

3.5. Efects of atropine or tetrodotoxin on the paxilline-induced stimulation of urinary bladder motility

The proposal (Selala et al., 1991) that tremorgenic alkaloids promote the release of acetylcholine from nerve

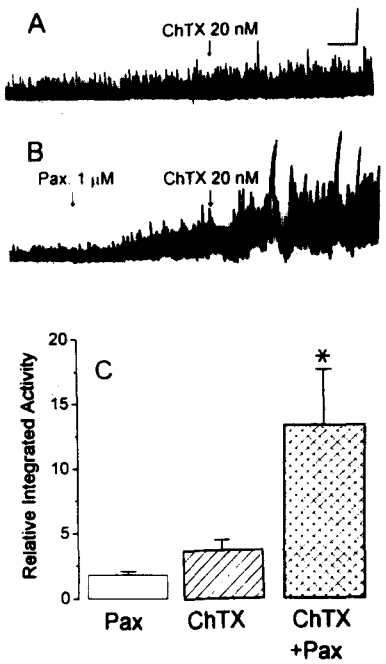

Fig. 4. Effect of paxilline and charybdotoxin on the contractility of guinea-pig urinary bladder strips. (A,B) Isometric tension recordings from two adjacent strips from the same bladder, exposed to 20 nM charybdotoxin (ChTX) in the absence (A) or in the presence (B) of paxilline (Pax; 1 mM), which was applied 20 min before the challenge with charybdotoxin. Calibration bars: horizontal, 5 min; vertical, 1 g. (C) Integrated mechanical activity data from 12 bladder strips, studied with the protocol depicted in A and B. The bars correspond to the relative integrated activity during exposure to 1 mM paxilline, or to 20 nM charybdotoxin in the absence or in the presence of 1 mM paxilline. * $P < 0.05$ for the difference between the effects of each BK_{Ca} blocker vs. the effects observed in the presence of both blockers.

terminals led us to investigate the effects of a possible functional interaction between paxilline and atropine on the myogenic activity of guinea-pig detrusor muscle. Atropine (100 nM) fully reversed the stimulatory effect of carbachol (100 nM), but did not affect the paxilline-induced enhancement of myogenic activity (Fig. 5). This effect of paxilline was also unaffected by tetrodotoxin (10 μM; Fig. 5).

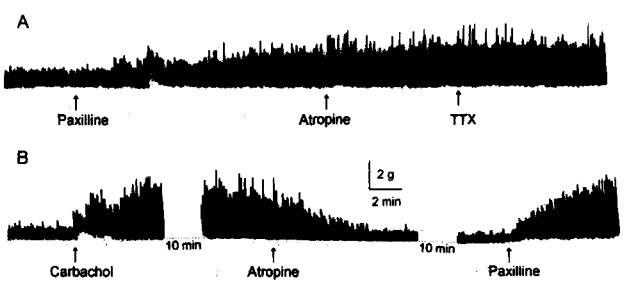

Fig. 5. Effect of atropine and of tetrodotoxin (TTX) on the paxilline-induced stimulation of the contractility of guinea-pig urinary bladder strips. A and B show isometric tension recordings from two adjacent strips taken from the same bladder, initially exposed to either 10 mM paxilline (A) or 10 mM carbachol (B). (A) The paxilline-induced stimulation of myogenic activity was not affected by addition of atropine (0.1 mM) or TTX (10 mM) to the bathing medium. (B) Atropine (0.1 mM) fully reversed the carbachol-induced contratility (B) but did not prevent the contractile response to paxilline then added to the bathing medium.

4. Discussion

This is the first systematic study of the effects of the tremorgenic indole alkaloids, paspalitrem-C and paxilline, on the spontaneous contractile activity of a variety of mammalian smooth muscle tissues. Previously, Selala et al. (1991), reporting that paxilline and two other indole alkaloid fungal mycotoxins, penitrem-B and verruculogen, increased the contractions elicited by electric field stimulation of isolated guinea-pig ileum, ascribed this effect to enhanced acetylcholine release from nerve terminals. The present observation that atropine does not modify the stimulatory effect of paxilline on the spontaneous motility of guinea-pig bladder, indicates that this effect is not mediated by activation of muscarinic receptors in the detrusor muscle. The plausibility of this interpretation is supported by our results with tetrodotoxin, which also argue against the possible involvement of other neurotransmitters as mediators of the paxilline-induced stimulation of spontaneous motility. Knaus et al. (1994) revealed that verruculogen and penitrem, like paxilline and paspalitrem-C, are potent blockers of the smooth muscle BK_{Ca} channel. Thus, the contribution of smooth muscle BK_{Ca} channel inhibition to the contractile effects observed in the study of Selala et al. (1991) may have remained unrecognized.

The enhancement of the spontaneous motility of guinea-pig and rat urinary bladder, and of rat duodenum observed in the present study is consistent with the inhibitory effect of paxilline and paspalitrem-C on BK_{Ca} channels (Knaus et al., 1994). Indeed, BK_{Ca} channels are found in the surface membrane of rodent urinary bladder and the gastrointestinal tract, where they play a predominant role in the repolarizing phase of the action potential (Klöckner and Isenberg, 1985; Hu et al., 1989). Inhibition of BK_{Ca} conductance leads to prolongation of the action pontential and, consequently, to increased Ca^{2+}-influx across the sarcolemma during the excitation-contraction coupling process. We suggest that this mechanism accounts for the increased spontaneous myogenic activity of rodent bladder and gastrointestinal muscles elicited by paxilline and paspalitrem-C. A similar interpretation has been proposed for the enhacement of bladder and gastrointestinal motility during exposure to the potent peptidyl inhibitors of the BK_{Ca} channel, charybdotoxin and iberiotoxin (Suarez-Kurtz et al., 1991).

The present observation that paxilline potentiates charybdotoxin as a stimulant of the myogenic activity of guinea-pig bladder, represents an important functional correlate for the synergistic interaction between these two agents previously observed in binding experiments (Knaus

et al., 1994). In the latter study, paxilline was shown to enhance [^{125}I]CHTX binding to the BK_{Ca} channel through an allosteric mechanism in which the indole, diterpene, increased the affinity of peptide for its binding site in the pore. Thus, it would be expected that the potency of charybdotoxin as a smooth muscle contractile agent should be increased in the presence of paxilline. A similar functional manifestation of allosteric coupling between ligands for the benzothiazepine and dihydropyridine sites in the L-type Ca^{2+} channel has been detected in cardiac contractility measurements (Garcia et al., 1986).

Paxilline and paspalitrem-C failed to significantly modify the myogenic activity of guinea-pig portal vein, although BK_{Ca} channels have been described in this tissue. Suarez-Kurtz et al. (1991) reported that the guinea-pig portal vein was not responsive to either charybdotoxin or iberiotoxin, and ascribed this result to a predominance in the activity of K^+ currents other than those through BK_{Ca} channels during the repolarization phase of the action potential. If the assumption that the contractile effects of the tremorgenic alkaloids in smooth muscle are due to selective inhibition of BK_{Ca} channels is correct, it follows that an explanation similar to that used earlier could account for the present results with guinea-pig portal vein.

However, the tremorgenic alkaloids and the peptidyl blockers differ in their functional effects on rat portal vein, guinea-pig aorta and, to a lesser extent, guinea-pig tracheal rings. Thus, in contrast to charybdotoxin and iberiotoxin which consistently enhance the spontaneous myogenic activity of rat portal vein (Winquist et al., 1989) and elicit tension in guinea-pig aorta (Suarez-Kurtz et al., 1991), paspalitrem-C had no effect on either tissue, and paxilline was ineffective on portal vein and induced tension in only a third of the aorta preparations tested. In guinea-pig tracheal rings, paspalitrem-C had no effect, whereas paxilline (10 μM) induced a detectable tension in 7 out of 16 preparations. Although there are some discrepancies in the literature regarding the ability of charybdotoxin to elicit tension in this tissue (Suarez-Kurtz et al., 1991; Huang et al., 1993), it is significant that rings that failed to respond to paxilline (10 μM) developed little or no tension when challenged with charybdotoxin (100 nM).

The discrepancies in the pharmacological profiles of the tremorgenic indole alkaloids vs. the peptidyl blockers may be related to various factors, such as their relative potencies as BK_{Ca} channel blockers in intact tissues, and/or differences in site and mode of action of these compounds. For the reasons listed in Section 2, the maximum concentration of either paspalitrem-C or paxilline that could be achieved in the muscle chamber was 10 μM. It is possible that higher concentrations of these agents would stimulate the spontaneous contractility of rat portal vein and consistently induce tension in guinea-pig aorta and tracheal rings. Both the indole alkaloids, paxilline and paspalitrem-C, and the peptidyl toxins, charybdotoxin and iberiotoxin, block BK_{Ca} channel currents with K_d values in the low (2–10) nanomolar range (Garcia et al., 1991; Giangiacomo et al., 1992; Knaus et al., 1994). Although functional effects on smooth muscle motility can be obtained with nanomolar concentrations of the peptidyl blockers (Suarez-Kurtz et al., 1991), micromolar concentrations are required in the case of the indole alkaloids. This discrepancy most likely results from the distinct sites of action of the two groups of BK_{Ca} channel blockers: the peptidyl blockers act by binding in the external pore to occlude the ion conduction pathway (for a review, see Garcia-Calvo et al., 1993), while the indole alkaloids block BK_{Ca} channel activity most potently when they are added at the internal surface of the channel (Knaus et al., 1994). Thus, these mycotoxins must permeate accross the plasma membrane to fully exert their inhibitory effect on BK_{Ca} channel.

Unitary channel recordings from vascular smooth muscle membranes revealed that, in contrast to that by the peptidyl toxins, blockade of the BK_{Ca} channel by the indole alkaloids is weaker when the Ca^{2+} concentration at the internal face of the membrane is elevated, and the open channel probability is high (Knaus et al., 1994). This factor might be related to the reported modulation of these compounds' effects on rat urinary bladder by the basal myogenic activity (and underlying fluctuations in the myoplasmic Ca^{2+} concentration) of the muscle strips (DeFarias, 1993).

Through the utilization of molecular biological techniques, many different families of K^+ channels have recently been identified, and studies are underway to define the tissue distribution of these various channel types. However, relatively little is known about the physiological role of these channels in target tissues of interest, and whether their activity can be modulated for therapeutic benefit. The same can be said for most of the subtypes of K^+ channels that have only recently been identified. The discovery and pharmacological characterization of specific K^+ channel modifiers provide a very important means to resolve such issues.

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