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Pharmacological Properties of Pteleprenine, a Quinoline Alkaloid Extracted from *Orixa japonica*, on Guinea-pig Ileum and Canine Left Atrium

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Abstract

We have investigated the pharmacological properties of pteleprenine, a quinoline alkaloid, on contractile responses of the guinea-pig ileum and on inotropic responses of the canine left atrium.

Although pteleprenine (0·1–1 μM) had no effect on the contraction of the ileum induced by acetylcholine at 10 μM it significantly inhibited acetylcholine-induced contraction of the ileum. Pteleprenine (0·1–10 μM) reduced nicotine induced-contraction of the ileum in a concentration-dependent manner yet had no maximum relaxant effect even at a concentration of 10 μM. From Schild analysis the pA_2 of pteleprenine on the guinea-pig ileum was found to be 6·6. The contraction of the ileum induced by 10 μM 1,1-dimethyl-4-phenylpiperazinium, a specific agonist of nicotinic acetylcholine receptors, was concentration-dependently suppressed by 10 nM–10 μM pteleprenine. In contrast, 0·1–10 μM pteleprenine did not antagonize the acetylcholine- and nicotine-induced negative inotropic contractile responses of the canine left atrium. These results show that pteleprenine has inhibitory action against nicotinic acetylcholine receptors in the guinea-pig ileum but not in the canine left atrium. Our findings also suggest that pteleprenine might be a novel lead compound as a nicotinic receptor antagonist.

Pteleprenine, a quinoline alkaloid (Figure 1), was first isolated from the leafless shoots of *Ptelea trifoliata* (Rutaceae) (Korosi et al 1974). It is also present in the stems of *Orixa japonica* and this plant is used in Japan as the insecticide Wa-johzan for livestock (Chin Su New Medical College 1977). Although it is well known that Wa-johzan has beneficial effects against gastralgia, rheumatoid arthritis and colds, the pharmacological properties of pteleprenine, one of the main alkaloids of Wa-johzan, are still unclear. Interestingly, a previous study suggested that high concentrations of skimmianine, an analogue of pteleprenine, can induce non-specific depression of cardiovascular function (Cheng et al 1990). Furthermore, several quinoline alkaloids are known to inhibit 5-hydroxytryptamine-induced contraction of a variety of smooth muscles (Kishibayashi et al 1993). From these observations it is conceivable that pteleprenine might have a relaxant effect on cardiac and smooth muscles.

In this study, therefore, we investigated the effects of pteleprenine on acetylcholine- and nicotine-induced contractile responses of the guinea-pig ileum and on acetylcholine- and nicotine-induced negative inotropic responses of the canine left atrium.

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Materials and Methods

Drugs

Acetylcholine chloride, nicotine base and polyethylene glycol were from Wako Chemical Industries (Osaka, Japan) and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) was from Aldrich (WI).

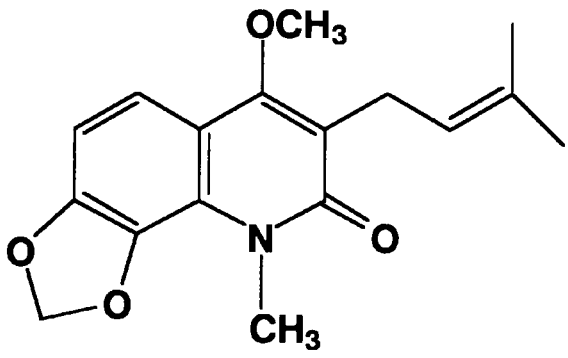

Figure 1. Structure of pteleprenine, 6-methoxy-9-methyl-7-(3-methyl-2-butenyl)-1,3-dioxo[4,5-*H*]quinoline-8(9*H*)-one.

Extraction and isolation of pteleprenine

Air-dried stems (1·0 kg) of *O. japonica* collected near Sendai, Japan in June 1996, were extracted twice for one day with methanol at room temperature. The extract (45 g) was chromatographed over silica gel (500 g) with *n*-hexane–ethyl acetate mixtures as mobile phase. The fraction eluted by 6 : 1 *n*-hexane–ethyl acetate (1·2 g) was repeatedly chromatographed on silica gel with *n*-hexane–acetone as mobile phase to give pteleprenine (20 mg) as a colourless powder. Spectroscopic data of pteleprenine were consistent with those published elsewhere (Funayama et al 1994); its chemical structure is shown in Figure 1.

Tissue preparation

All tissues were prepared according to methods previously described (Motomura et al 1990; Boselli & Grana 1995). Ileum was removed from male guinea-pigs, 300–400 g, after exsanguination. Segments of the ileum (2 cm) were mounted in 25-mL organ baths, containing modified Tyrode solution at 30°C, and suspended under 1 g tension. Left atrium was removed from previously anaesthetized mongrel dogs, 8–11 kg, of either sex. The left atrium was dissected and strips were mounted in 25-mL organ baths, containing modified Tyrode solution at 30°C, and suspended under 0·4 g tension. The atrial strips were electrically paced by means of platinum punctate electrodes (1 Hz, 1 ms duration, at twice the threshold voltage). The electrical pacing conditions were chosen to override any spontaneous rhythm resulting from pacemaker activity; only changes in contractile force were apparent.

Experimental protocol

Cumulative concentration–response curves for the agonist were obtained by dosing at 0·5 log unit (acetylcholine) or 1 log unit (nicotine) intervals in the absence or presence of pteleprenine. Serial higher concentrations of each agonist were applied to the organ bath either after 1 min if no response was observed or 1 min after the response to the concentration added reached a plateau. Nicotine-induced response of organs was usually achieved by once-repeated addition of the initial concentration administered. The tissues were equilibrated for 30–60 min (ileum) or 60–90 min (left atrium) before applying each agonist. The cumulative concentration–response curves for the agonist were obtained after 5 min (ileum) or 10 min (left atrium) incubation with solvent containing an amount of polyethylene glycol sufficient to dissolve the amount of pteleprenine used. The maximum effect on the contractile response of the ileum induced by each agonist was regarded as a 100% contraction. In the canine left atrium the maximum value was regarded as a 100% negative inotropic effect. After wash-out every 10 min, each concentration of pteleprenine was incubated for 5 min (ileum) or 10 min (left atrium), and the cumulative concentration–response curve for the agonist was repeated. Only one concentration of pteleprenine was tested for each preparation. The final concentration of poly(ethylene glycol) used in this study had no significant effect on the experimental results.

The contractile response of 10 μM DMPP was measured for three concentrations of pteleprenine (0·1, 1 and 10 μM) in the guinea-pig ileum. After 30–60 min equilibration, the ileum was exposed to 10 μM DMPP for 1 min after 5-min incubation with solvent containing sufficient poly(ethylene glycol) to dissolve the concentration of pteleprenine used. A 30–60-min recovery period was required before subsequent exposure to DMPP in the presence of pteleprenine. The medium was changed at least three times during equilibration and recovery periods. Pteleprenine was incubated for 5 min and a similar response to DMPP was traced for 1 min. Only one concentration of pteleprenine was tested on each preparation.

Statistical evaluation

The results are expressed as mean ± s.e. Statistical analysis was performed by unpaired Mann-Whitney *U*-test and $P < 0.05$ was regarded as statistically significant.

Results

Effects of pteleprenine on acetylcholine- and nicotine-induced contractions of the guinea-pig ileum

Acetylcholine (1 nM–10 μM) induced concentration-dependent contraction of the guinea-pig ileum. Pteleprenine at concentrations of 0·1 and 1 μM had

no effect on this contraction but at a concentration of 10 μM antagonized acetylcholine-induced contraction of the ileum, as shown in Figure 2. The maximum relaxant effect of 10 μM pteleprenine was 56·3 ± 18·5%. Nicotine (0·1–100 μM) also induced concentration-dependent contraction of the guinea-pig ileum. Pteleprenine (0·1–10 μM) inhibited the nicotine (1–100 μM)-induced contraction of the guinea-pig ileum in a concentration-dependent manner, as shown in Figure 3. However, pteleprenine at a higher concentration of 10 μM partially inhibited contraction of guinea-pig ileum induced by 100 μM nicotine. The Schild plot appeared to be fitted by a straight regression line and gave a pA_2 value of approximately 6·6.

To further confirm the inhibitory effect, we investigated the effect of pteleprenine on DMPP (a specific agonist for nicotinic acetylcholine receptor)-induced contraction of the guinea-pig ileum. The contractile response of the ileum nearly reached the maximum value at 30 μM DMPP and the median effective dose was 1·1 μM, as shown in Figure 4A. Pteleprenine (10 nM–10 μM) inhibited the contraction of the ileum induced by 10 μM DMPP in a concentration-dependent manner. In particular, 1 and 10 μM pteleprenine significantly antagonized the contraction of the ileum produced by 10 μM DMPP, as shown in Figure 4B.

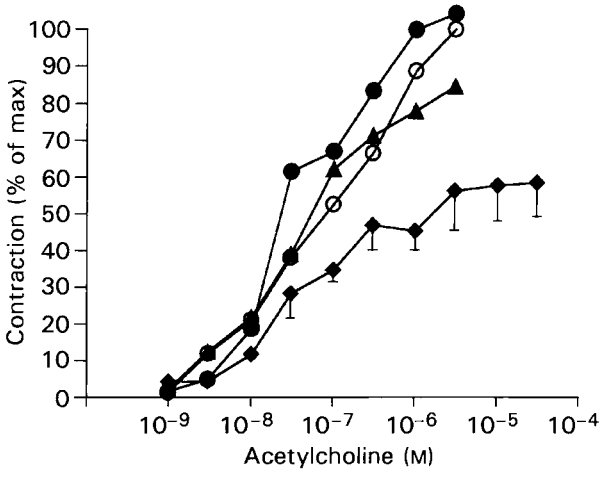

Figure 2. Antagonistic effect of pteleprenine on the acetylcholine-induced contractile responses of the guinea-pig ileum. ○ Control; ● 10^{-7} M pteleprenine; ▲ 10^{-6} M pteleprenine; ◆ 10^{-5} M pteleprenine. Data are means ± s.e. from five experiments. Pteleprenine was added 10 min before the agonist.

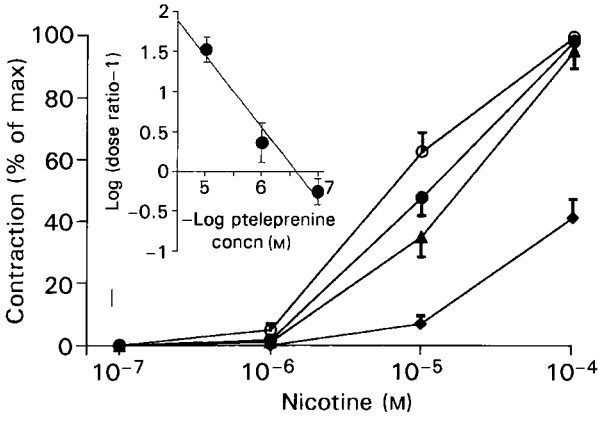

Figure 3. Antagonistic effect of pteleprenine on the nicotine-induced contractile responses of the guinea-pig ileum. ○ Control; ● 10^{-7} M pteleprenine; ▲ 10^{-6} M pteleprenine; ◆ 10^{-5} M pteleprenine. Data are means ± s.e. from four experiments. Pteleprenine was added 10 min before the agonist.

Effect of pteleprenine on the acetylcholine- and nicotine-induced contraction of the canine left atrium

In the canine left atrium, acetylcholine (1 pM–10 μM) elicited the negative inotropic effect in a concentration-dependent manner. This negative inotropic effect was not influenced by pteleprenine (0·1–10 μM), as shown in Figure 5A. Nicotine (10 pM–100 μM) also elicited the negative inotropic effect in the canine left atrium in a concentration-dependent manner. Pteleprenine (0·1–10 μM) did not antagonize this nicotine-induced negative inotropic effect in the atrium, as shown in Figure 5B.

Discussion

This study provides evidence that pteleprenine, a quinoline alkaloid, inhibited nicotine-induced contraction of the guinea-pig ileum in a concentration-dependent manner whereas the contractile effect of acetylcholine was partially modified by this compound only at higher concentrations. In contrast, pteleprenine did not antagonize the negative inotropic effect in the canine left atrium produced by acetylcholine or nicotine. These findings suggest that pteleprenine might act as a nicotinic cholinergic receptor antagonist and have organ specificity between the atrium and ileum.

Pteleprenine at 0·1 μM inhibited nicotine-induced contraction of guinea-pig ileum whereas the contractile effect of the ileum induced by acetylcholine was not antagonized by pteleprenine in a concentration-dependent manner. Furthermore, the negative inotropic effect of acetylcholine or nicotine in the canine left atrium was unaffected by pteleprenine. It is known that the canine left atrium and ileum have parasympathetic ganglia, and nicotinic acetylcholine receptors are predominantly located on the left atrium (Michael et al 1979). However, it is presently unclear whether the nicotinic acetylcholine receptors in the guinea-pig ileum are similar to those in the canine left atrium. Furthermore, at least ten subtypes of nicotinic

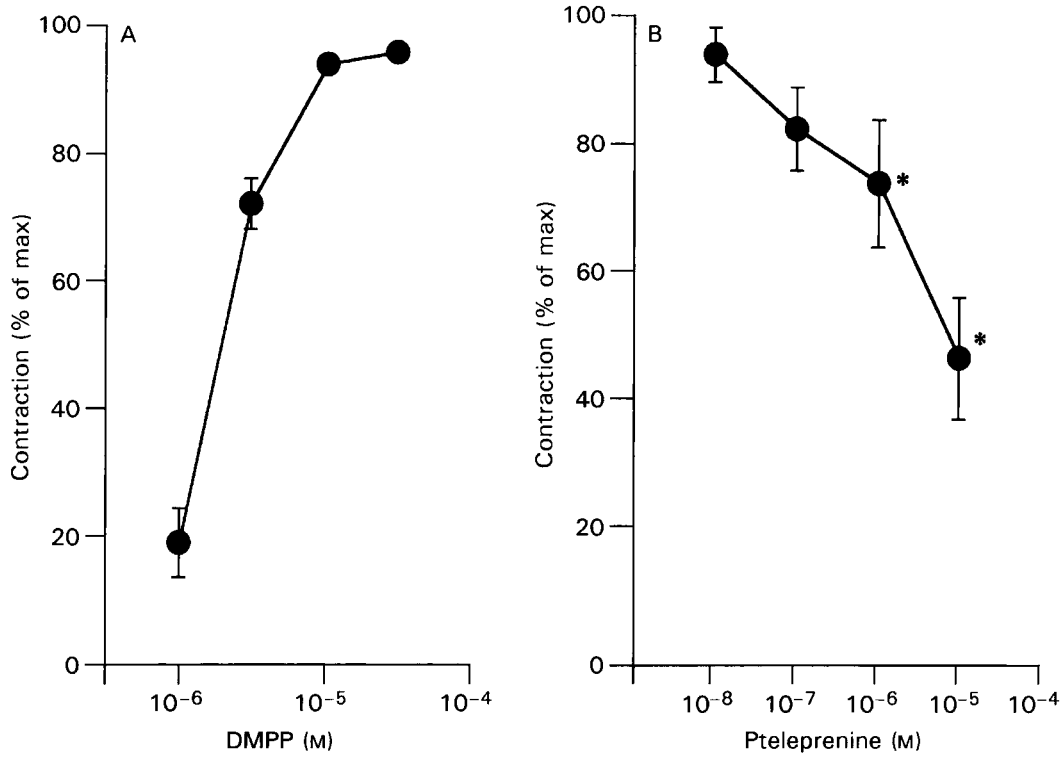

Figure 4. DMPP-induced contractile responses of the guinea-pig ileum (A) (n = 3) and antagonistic effects of pteleprenine on the contractile responses induced by 10^{-5} M DMPP in the guinea-pig ileum (B) (n = 4). *$P < 0.05$, significantly different from result obtained with 10^{-8} M pteleprenine.

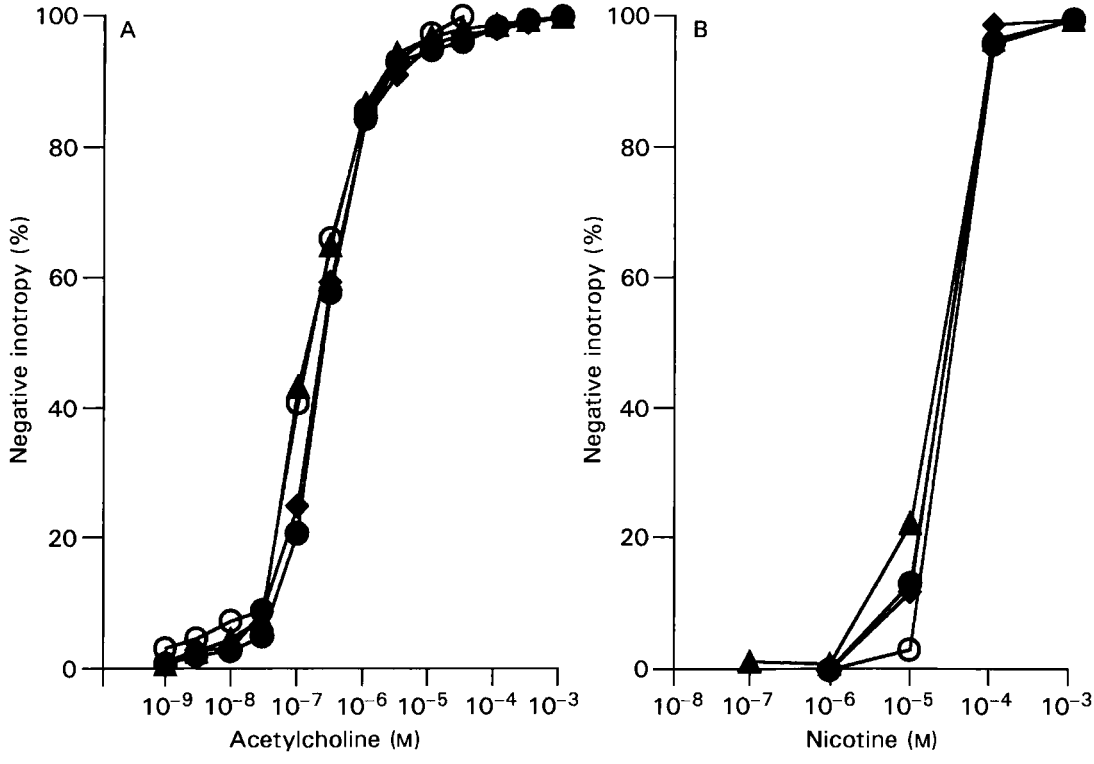

Figure 5. Antagonistic effects of pteleprenine against (A) acetylcholine- and (B) nicotine-induced negative inotropic effects on the canine left atrium. ○ Control; ● 10^{-7} M pteleprenine; ▲ 10^{-6} M pteleprenine; ● 10^{-5} M pteleprenine. Data are means ± s.e. from three experiments. Pteleprenine was added 10 min before the agonist.

acetylcholine receptor have been identified (Kerr et al 1995). A recent interesting study reported different responses of nicotinic acetylcholine receptors among species (Lukas et al 1995). These observations seem to suggest that nicotinic acetyl-choline receptor subunits in the guinea-pig ileum might be different from those in the canine left atrium. Therefore, our findings suggest that pteleprenine might be a compound with organ specificity.

To further test the selectivity of the antagonism, we also examined the effect of pteleprenine on DMPP-induced contraction of the guinea-pig ileum. DMPP-induced contraction of the ileum was inhibited by pteleprenine in a concentration-dependent manner. In particular, pteleprenine at concentrations of 1 and 10 μM significantly antagonized the contractile response of the ileum induced by 10 μM DMPP. This finding also suggests that pteleprenine has inhibitory properties on nicotinic acetylcholine receptors in the guinea-pig ileum.

To the best of our knowledge, little is known about the antagonistic effect of quinoline alkaloids on nicotinic acetylcholine receptors. This study demonstrates that the affinity of pteleprenine for nicotinic acetylcholine receptors in the guinea-pig ileum ($pA_2 = 6{\cdot}6$) is stronger than that of hexamethonium ($pA_2 = 5{\cdot}5$), a prototype autonomic ganglion blocker (Yamada et al 1987). Therefore, the current study suggests that pteleprenine might be regarded as a novel tool for exploring the physiological and pathological roles of selective autonomic ganglion blockers, although further studies are required to clarify our findings.

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