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Synthesis and Smooth Muscle-Selective Relaxant Activity of a Piperidine Analogue: 1-(4'-Fluorophenacyl)-4-Hydroxy-4-Phenyl-Piperidinium Chloride

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The antispasmodic and vasodilator activities of a newly synthesized piperidine derivative (1-(4'-fluorophenacyl)-4-hydroxy-4-phenyl-piperidinium chloride) were studied *in vitro*. The test compound exhibited a dose-dependent relaxant effect on the spontaneous and K^+ (75 mM)-induced contractions of isolated rabbit jejunum with respective EC_{50} values of 0.01 mM (0.01-0.02, 95% CI) and 0.30 mM (0.17-0.56). The Ca^{++} channel blocking (CCB) activity was confirmed when the test compound (0.1-0.2 mM) shifted the Ca^{++} dose-response curves to the right, similar to that produced by verapamil (0.1-1.0 μM), a standard CCB. In the isolated rabbit aorta, the test compound showed a dose-dependent vasodilator effect on K^+ (75 mM)-induced contractions with an EC_{50} value of 0.08 mM (0.02-0.26) while also suppressed the norepinephrine (1 μM) control peak responses with EC_{50} value of 0.08 mM (0.05-0.13, n=5). When tested in Langendorff perfused rabbit heart preparation, the test compound exhibited a negligible inhibitory effect on the rate or force of atrial and ventricular contractions when tested up to 5 mM. The results show smooth muscle-selective relaxant effect of the test compound on intestinal and vascular preparations mediated possibly via blockade of voltage and receptor-operated Ca^{++} channels.

Key words: Piperidine analogue, Spasmolytic, Ca^{++} antagonist, Vasodilator, Smooth muscle-selectivity

INTRODUCTION

4-phenylpiperidine was the first synthetic piperidine analogue that was reported as a spasmolytic agent (Eisleb and Schaumann, 1939). Since then, majority of studies conducted with piperidine analogues have been in neuronal tissues in an attempt to search for better analgesics (Pugsley, 2002; Saify *et al.*, 2005). The calcium channel blocking (CCB) activity of piperidine derivatives has not been widely reported. Loperamide is a well-known therapeutic agent which displays pharmacological calcium antagonism in smooth muscles and contains a piperidine nucleus as well. These calcium antagonist actions may be responsible in part for its antidiarrhoeal effects (Reynolds *et al.*, 1984). Moreover, phenacyl derivatives of 4-hydroxy-piperidine have been synthesized and found to exhibit spasmolytic activity (Saeed *et al.*, 1998) but the calcium antagonist activity was not reported.

The test compound in this investigation is a chloride salt of 4-hydroxy-4-phenyl-piperidine in which fluorophenacy has been substituted. The experiments have been designed to evaluate the effect of the test compound in intestinal and vascular smooth muscles and in the Langendorff perfused whole heart preparation. The test compound exhibited CCB activity selectively in smooth muscle preparations with only a mild inhibitory effect on the cardiac tissues.

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MATERIALS AND METHODS

Synthesis of the test compound

Equimolar quantity of 4-hydroxy-4-phenylpiperidine chloride and 1-(4-fluoro)-phenacyl-substituted piperidine were dissolved in acetone (30 mL) and refluxed on a water bath and the reaction was continuously monitored by TLC using the solvent system $CHCl_3$-MeOH in the ratio of 9:1.When all the starting material changed into product, the resulting solid material or the precipitate was collected by filtration and thoroughly washed to remove traces of reactant. It was then dissolved and re-crystallized from ethyl alcohol: yield 54%, m.p. 198-200°C.

Spectral data:

UV (MeOH) 1max nm: 310,247 and 205; IR (KBr) n/cm: 3325, 2950, 2775, 1680, 1590, 1225, 980, 690; EIMS m/z (%) 313 (M^+-HCl, $C_{19}H_{20}FNO_2$, 1), 190 (100), 176 (18), 171 (15), 159 (14), 123 (5), 104 (14), 91 (11), 82 (11), 77 (16); ^{1}H-NMR. (CD3 OD, 300 MHz) δ: 8.00 (2H. d. J=7.98 Hz, H-2', 6'). 7.68 (2H, d, J=7.98 Hz, H-3', 5'), 7.50 (2H, dd, J=8.37, 2.04 Hz, H-2", 6"), 7.37(2H, t, J=7.14, Hz, H – 3", 5"), 7.28 (1H, dd, J= 6.90, 2.04 Hz, H-4"), 6.02 (2 H, s, H-α), 3045 (2H, dt, J=12.77, 4.32 Hz, H-2a,6a), 302 (2H, dt, J=12.77, 2.20 Hz, H- 2b,6b), 2.22 (2H, ddd, J=18.09, 12.77, 4.32 Hz H-3a,5a), and 1.92 (2H,ddd, J=18.09, 4.32, 2.20 Hz, H-3b,5b). $C_{19}H_{21}ClFNO_2$ Formula Weight: 349.84; Calc % $C_{65.84}$ $H_{6.05}$ $N_{4.00}$, Found % $C_{65.18}$ $H_{6.00}$ $N_{3.94}$.

Drugs and chemicals

Acetylcholine (ACh), norepinephrine (NE) and verapamil were obtained from Sigma Chemical Company, St. Louis, MO, U.S.A., while heparin injections BP were purchased from Rotex Medica, Trittau, Germany. The following chemicals were used to make the physiological salt solutions: potassium chloride (Sigma Chemical Company, St. Louis, MO, U.S.A.), calcium chloride, glucose, magnesium chloride, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, sodium dihydrogen phosphate (E.Merck, Darmstadt, Germany), and ethylenediaminetetra-acetic acid (EDTA) from BDH Laboratory Supplies, Poole, England. Stock solutions of all the chemicals were made in saline fresh on the day of the experiment.

Animals

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996). Local male rabbits (1 kg) used in the study were housed in the animal house of the Aga Khan University under a controlled environment (23-25°C). Animals were given tap water *ad libitum* and a standard diet consisting of (g/kg): flour 380, fibre 380, molasses 12, NaCl 5.8, nutrivet L 2.5, potassium metabisulphate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150.

Isolated rabbit jejunum

Experiments were performed as described earlier (Gilani and Cobbin, 1986). Segments of rabbit jejunum tissue 2 cm long were suspended in 10 mL tissue baths containing Tyrode's solution, aerated with a mixture of 95% oxygen and 5% carbon dioxide (carbogen) and maintained at 37°C. The composition of Tyrode's solution in mM was: KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8, and glucose 5.55. Intestinal responses were recorded isotonically using Harvard student oscillographs and isotonic transducers. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. Under these conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing of relaxant (spasmolytic) activity directly with out the use of an agonist.

Determination of Ca^{++} antagonist activity in rabbit jejunum

To assess whether the spasmolytic activity of the test compound was mediated through CCB, K^+ (75 mM) was used to depolarize the preparations (Farre *et al.*, 1991). High K^+ (75 mM) was added to the tissue bath, which produced a sustained contraction. The test compound was then added in a cumulative fashion to obtain concentration-dependent inhibitory responses. The relaxation of intestinal preparations, precontracted with K^+ (75 mM) was expressed as percent of the control response mediated by K^+. Contraction of smooth muscle induced by K^+ is known to be mediated, *via* influx of Ca^{++} from extracellular fluid and a substance, which inhibits this contraction, is considered to act through blockade of Ca^{++} channels (Bolton, 1979).

To confirm the Ca^{++} antagonist activity of the test compound, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca^{++}-free Tyrode's solution containing EDTA (0.1 mM) for 30 min in order to remove Ca^{++} from the tissues. This solution was further replaced with K^+-rich and Ca^{++}-free Tyrode's solution, having the following composition (mM): KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55, and EDTA 0.1. Following an incubation period of 30 min, control dose-response curves (DRCs) of Ca^{++} were obtained. When the control DRCs of Ca^{++} were found super-imposable (usually after two cycles), the tissue was pretreated with the test compound for 60 min to test the possible CCB effect. The DRCs of Ca^{++} were reconstructed in the presence of different concentrations of the test compound while verapamil was used as a positive control.

Isolated rabbit aorta

Rabbits were sacrificed and the descending thoracic aorta was removed and cut into 2-3 mm wide rings which were individually mounted in 20 mL tissue baths containing Kreb's-Henseliet solution (composition in mM: NaCl 11.50, KCl 4.70, $CaCl_2$ 2.50, $NaHCO_3$ 25.0, $MgSO_4 \cdot 7H_2O$ 1.50, $K_2H_2PO_4 \cdot 2H_2O$ 1.20, and glucose 11.0) at 37°C and aerated with carbogen gas. A resting tension of 2 g was applied to each tissue and an equilibrium period of 1 h was allowed before any experimentation. The changes in isometric tensions of the rings were measured *via* a force-displacement transducer (FT-03) using a Grass Model 7 Polygraph. Following an equilibrium period of 1 h, the tissues were stabilized with a fixed dose of NE (1 μM). The tissues were considered stable only when similar responses were obtained from the repeated doses of NE (1 μM). Effect of the test compound was first determined on the resting baseline of the tissue to see if it has any vasoconstrictor effect. Later it was tested for any ability to relax the high K^+ (75 mM)-induced contractions or control NE (1 μM) peak responses. The ability of the extract to relax K^+ (80 mM)-induced contractions would indicate L-type voltage-dependent CCB mode of vasodilation while inhibition of the NE-peak responses would signify the blockade of the Ca^{++} influx through the receptor-operated Ca^{++} channels (Karaki, 2004). Procedure for the latter possibility involved incubating the control NE responses with increasing doses (0.005-1.0 mM) of the test compound for 1 h.

Langendorff perfused rabbit heart

Whole hearts were obtained from healthy rabbits (male, 1 kg). Heparin (5000 I.U) was injected (i.p) 1 h prior to isolation of the whole hearts. After cervical dislocation, hearts were excised rapidly and mounted on Langendorff apparatus as quickly as possible. Kreb's-Henseliet solution perfused the heart retrogradely, aerated by carbogen at thermostatically controlled temperature (37°C) with pH of 7.4. Atrial and ventricular activities were recorded simultaneously and separately by two different Harvard isotonic transducers. Approximately 60 min were allowed to each heart to adapt to the new environment and to exhibit sino atrial nodal pattern of the cardiac activity. Any heart showing an abnormal pattern was discarded. After taking 10 min of equilibrium period, the test compound was added in ascending order. For each dose, 10 min were allowed to achieve the peak effect. Changes in atrial and ventricular activity were calculated when maximal effect persisted for 5 min or more (Staff Department of Pharmacology, Edinburgh, 1970).

Statistical analysis

All the data expressed are mean ± standard error of mean (SEM, n = number of experiments). Concentration-response curves were analyzed by non-linear regression (GraphPAD program, GraphPAD, San Diego, CA, U.S.A.).

RESULTS AND DISCUSSION

When tested on the spontaneously contracting isolated rabbit jejunum, the test compound caused a dose-dependent (0.003-0.1 mM) relaxant effect (Fig. 2) with an EC_{50} value of 0.01 mM (0.01-0.02, 95% CI, n=5). When tested on the high K^+ (75 mM)-induced contractions, the test compound exhibited a dose-dependent (0.01-0.5 mM) inhibition (Fig. 2) with an EC_{50} value of 0.30 mM (0.17-0.56, n=5). The contractions of smooth muscles, including that of rabbit jejunum, are dependent upon an increase in the cytoplasmic free Ca^{++}, which activates the contractile elements (Karaki, 2004). While contraction induced by high K^+ is dependent upon the entry of Ca^{++} into the cells through the voltage-operated Ca^{++} channels and thus inhibition of high K^+-induced contraction is due to the result of blocked Ca^{++} entry through these channels (Bolton, 1979), a characteristic of Ca^{++} antagonists. The interaction with Ca^{++} channels was further studied in jejunum, which is known to be quick in responding to

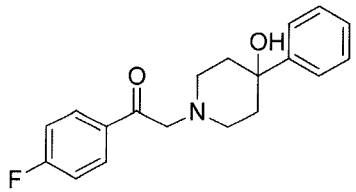

Fig. 1. Figure showing the chemical structure of the test compound: 1-(4′-fluorophenacyl)-4-hydroxy-4-phenyl-piperidinium chloride

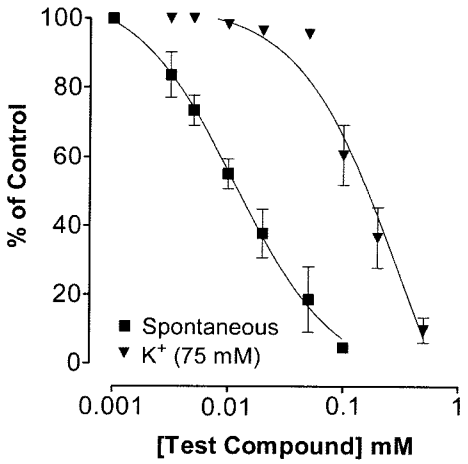

Fig. 2. Dose-response curves showing the dose-dependent spasmolytic effect of the test compound in spontaneous and high K^+ (75 mM)-contracted isolated rabbit jejunum (values shown are mean ± SEM, n=5.)

spasmolytic activity. The test compound dose-dependently (0.1-0.2 mM, n=4) shifted the Ca^{++} dose-response curves to the right (Fig. 3A), similar to that produced by verapamil (0.1-1.0 μM, n=3; Fig. 3B), a standard CCB (Bolton, 1979; Godfraind, 1987), thus confirming the CCB activity. However, the relatively high potency of the test compound in relaxing the spontaneously contracting rabbit jejunum compared to the effect on high K^+-induced contraction, points towards an additional spasmolytic mechanism that might also be present in the compound which could not be identified here in this study. If the compound only had the Ca^{++} antagonist activity, then it would have been more potent in relaxing the high K^+ than the spontaneous contractions.

Similar to the test compound, loperamide is a clinically used piperidine-like antidiarrhoeal drug which mediates its intestinal relaxant effect through a combination of different mechanisms including calcium channel blockade (Reynolds *et al.*, 1984). Different other 4-phenyl piperidine analogs have also been synthesized and investigated in search of better antidiarrhoeal agents with calcium antagonist activity. This was the reason for conducting this investigation, but this is only a preliminary study and a lot more work is needed to come to a definitive conclusion about a possible clinical use of this compound in intestinal spasmodic conditions such as diarrhoea.

Keeping in mind the proven use of CCBs in cardiovascular disorders such as hypertension (Godfraind *et al.*, 1986; Triggle, 1992), the test compound was tested in isolated aorta and whole heart preparations. The test compound was found devoid of any contractile effect on the resting baseline however, when tested on high K^+ (75 mM)-induced contractions, it showed a dose-dependent (0.001-0.1 mM) vasodilator effect (Fig. 4A) with an EC_{50} value of 0.08 mM (0.02-0.26, n=5) thus reiterating the already observed CCB activity in the jejunum. Likewise, the test compound dose-dependently (0.05-0.5 mM) inhibited the control peak responses of NE (1 μM) after pretreating the peaks with each of the test compound dose for 1 h (Fig. 4B) with an EC_{50} value of 0.08 mM (0.05-0.13, n=5), indicating inhibition of receptor-operated Ca^{++} channels as well. This suggested non-specific Ca^{++}-antagonist activity of the test compound on voltage- and receptor-operated channels (Karaki, 2004). The vasodilator activity of the test compound is in accordance with the earlier findings of other piperidine derivatives shown to have vasodilator and hypotensive activities (Clark *et al.*, 1983; Takai *et al.*, 1985).

In Langendorff perfused rabbit heart, the test compound was found devoid of any clear inhibitory effect even when

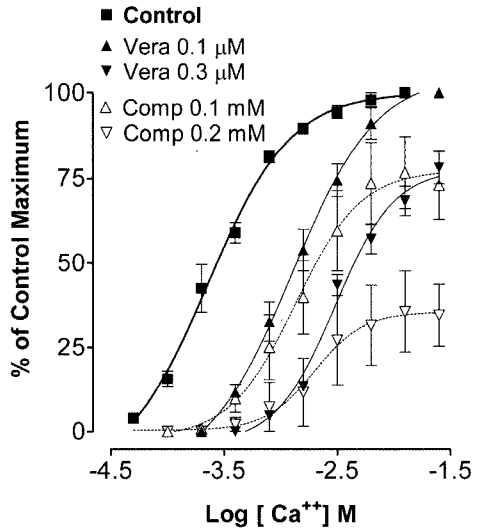

Fig. 3. Dose-response curves showing the inhibitory effect of increasing doses of the test compound (Comp, hollow symbols) and verapamil (Vera, black symbols) on Ca^{++} concentration-response curves, constructed in a Ca^{++}-free medium, in rabbit jejunum preparations (values shown are mean ± SEM, n=3-4.)

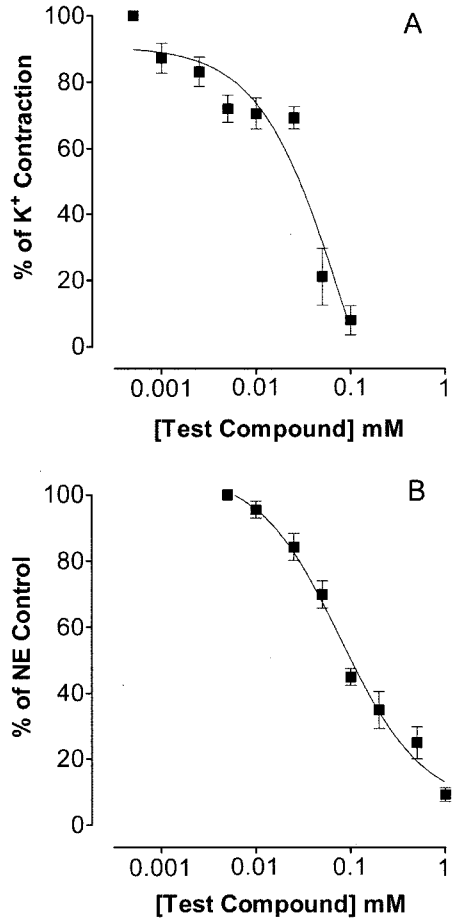

Fig. 4. Figure showing the inhibitory effect of increasing doses of the test compound on [A] high K^+ (75 mM)-induced contractions and [B] norepinephrine (NE, 1 μM) control peak responses in isolated rabbit aorta (values shown are mean ± SEM, n=5.)

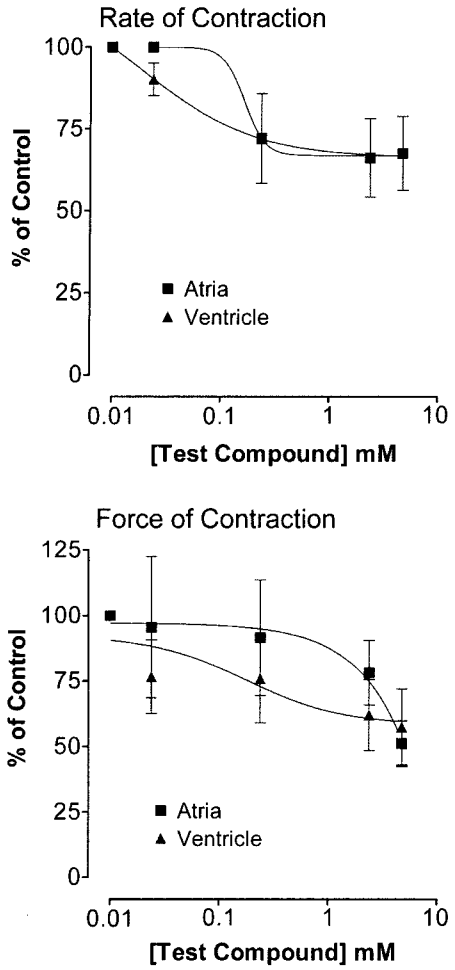

Fig. 5. Dose-response curves showing the effect of increasing doses of the test compound on rate and force of atrial and ventricular contractions of rabbit whole heart perfused preparation (values shown are mean ± SEM, n=5.)

tested up to 5 mM on the rate (Fig. 5A) and force (Fig. 5B) of artial and ventricular contractions. This showed that the test compound is specific in its relaxant effect for the smooth muscle preparations (intestinal and vascular) mediated possibly *via* CCB. We have earlier reported activity of some pure compounds that had a similar smooth muscle-selective relaxant profile (Gilani *et al.*, 2005). There is sufficient evidence of heterogenicity of Ca^{++} channels (Koike *et al.*, 1992) and different CCBs exhibit selectivity for different organ systems (Vanhoutte, 1981). For example, nifedipine is considered vascular selective with negligible effect on heart (Farre *et al.*, 1991).

The results showed intestinal spasmolytic and vasodilator activities of the test compound mediated possibly through CCB though an additional spasmolytic mechanism cannot be ignored. The test compound was unable to exhibit any significant inhibitory effect on the cardiac tissues thus indicating its smooth muscle-selective relaxant behaviour.

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