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Synthesis and Biological Evaluation of 1-(2′, 4′-dimethoxyphenacyl)-4-acetyl-4-phenylpiperidinium bromide in Intestinal and Cardiovascular Tissues*

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Abstract: 1-(2′, 4′-dimethoxyphenacyl)- 4-acetyl-4-phenylpiperidinium bromide is a synthetic piperidine compound which was screened in isolated intestinal and aortic smooth muscle and Langendorff's heart preparations. The test compound exhibited dose-dependent spasmolytic effect on the spontaneous and high K^+ (75 mM) contracted isolated rabbit jejunum with respective EC_{50} values of 0.08 mM (0.05-0.13, 95% CI) and 0.12 mM (0.06-0.21). The Ca^{++} channel blocking (CCB) activity was confirmed when the test compound (0.05-0.1 mM) shifted the Ca^{++} dose-response curves to the right, similar to that produced by verapamil (0.3-1.0 μM), a standard CCB. In the isolated rabbit aorta, the test compound showed a dose-dependent vasodilator effect on high K^+ (75 mM)-induced contractions with an EC_{50} value of 0.04 mM (0.02-0.08) while also suppressed the norepinephrine (1 μM) peak responses with an EC_{50} value of 0.04 mM (0.03-0.06). When tested in Langendorff perfused rabbit heart preparation, it exhibited a negative inotropic effect in the ventricles with EC_{50} value of 0.59 mM (0.04-8.86) with negligible effect on the rate of ventricular contractions. The results show inhibitory effects of the test compound on intestinal and vascular smooth muscle preparations and a cardio-suppressant effect, possibly mediated via blockade of voltage and receptor-operated Ca^{++} channels.

Key words: Piperidine analogue, spasmolytic, Ca^{++} antagonist, vasodilator, specific inotropic

Introduction

Piperidine derivatives are known to exhibit antihypertensive activities (Maillard *et al*., 1972; Clark *et al*., 1983). According to recent reports of pharmacological piperidine nucleus in their skeletons (Saeed *et al*., 1997, 1998; Saify *et al*., 2005). This led effects of substituted piperidines, there is still an increasing interest in synthesizing and evaluating new compounds having us to synthesize the test

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compound: 1-(2′, 4′-dimethoxyphenacyl)-4-acetyl-4-phenylpiperidinium bromide, belonging to phenylpiperidine class (North, 1986) and this was evaluated on isolated intestinal and vascular smooth muscles as well as isolated Langendorff perfused heart preparation. The results showed spasmolytic, vasodilator and specific negative inotropic activities of the test compound possibly mediated via calcium channel blockade (CCB).

Materials and Methods

Synthesis of the Test Compound

Equimolar quantity of 4-acetyl-4-phenylpiperidine and 2′, 4′-dimethoxyphenacyl bromide were dissolved in acetone (Fig. 1) 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 recrystallized from ethyl alcohol: yield 67%, m.p. 224-226°C.

Spectral Data

1H-NMR ($CDCl_3$, 400 MHZ) δ: 7.88 (1H. d. J = 8.78 Hzm H-6'), 7.31 (5H, m, H, 2″-6″), 6.53 (1 H, dd, J = 8.78. 2.29 Hz, H-5″). 6.41 (1H, d, J = 2.29 Hz, H-3′), 6.40 (2H, s, H-α). 3.87 (3H, s, Ar-OCH_3), 3.84 (3H, s, Ar-OCH_3), 3.83-3.80 (4H, m, H-2.6), 2.57-2.40 (4H, m, H-3.5) and 1.93 (3H s, $COCH_3$, C-4).

EIMS m/z (relative int.,%): 381 (M + -HBr, C 23 H27 NO_4, 1), 336 (4), 259 (3), 217 (15), 216 (100), 202 (18), 173 (6), 165 (30), 159 (7), 129 (6) and 82 (37).

IR Vmax (KBr) cm^{-1}: 2800, 2500, 1570, 1430, 1240 and 940.

UV λmax MeOH) nm: 351, 256 and 203.

$C_{23}H_{28}BrNO_4$, Formula Weight: 467.13

Drugs and Chemicals

Acetylcholine (ACh), norepinephrine (NE) and verapamil were obtained from Sigma Chemical Company, St. Louis, MO, USA 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, USA), 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 (around 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.

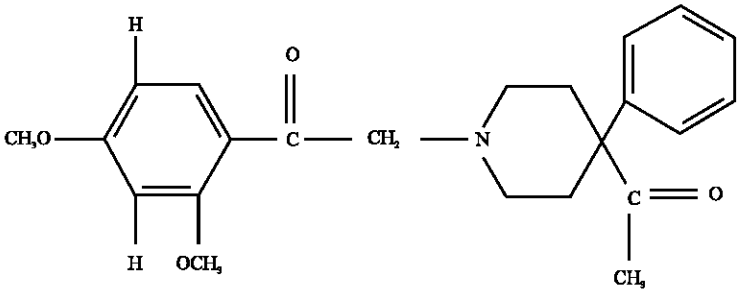

Fig. 1: The chemical structure and formula of the test compound1-(2′, 4′-dimethoxyphenacyl)-4-acetyl-4-phenylpiperidinium bromide

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, $MgSO_4.7H_2O$ 1.50, $K_2H_2PO_4.2H_2O$ 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 (0.01-0.5 mM) of the test compound for 1 h.

Langendorff Perfused Rabbit Heart

Whole hearts were obtained from healthy rabbits (male, 1 kg). Heparin (5000 IU) was injected (ip) 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. Ventricular activity was recorded by 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. Heart showing abnormal patterns were 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 ventricular activity were calculated when maximal effect persisted for 5 min or more (Staff Department of Pharmacology, University of Edinburgh, 1970).

Statistical Analysis

All the data expressed are mean±standard error of mean (SEM, n = number of experiments) and the median effective concentrations (EC_{50} values) with 95% confidence intervals (CI). Concentration-response curves were analyzed by non-linear regression (GraphPAD program, Graph PAD, San Diego, CA, USA).

Results and Discussion

When tested on the spontaneously contracting isolated rabbit jejunum, the test compound caused a dose-dependent (0.005-0.2 mM) relaxant effect (Fig. 2) with an EC_{50} value of 0.08 mM (0.05-0.13, 95% CI, n = 4). To elucidate the possible mechanism of this spasmolytic effect, the test compound was tested on high K^+ (75 mM)-induced contractions. It exhibited a dose-dependent (0.01-0.5 mM) inhibition (Fig. 2) of these induced contractions with an EC_{50} value of 0.12 mM (0.06-0.21, n = 4). 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

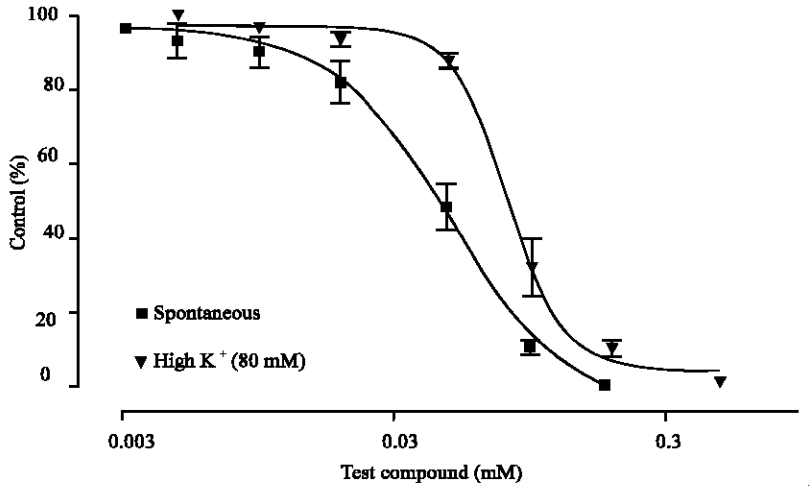

Fig. 2: Dose-response curves showing the dose-dependent spasmolytic effect of the test compound in spontaneous and high K^+ (75 mM)-contracted isolated rabbit jejunum (values shown are mean±SEM, n = 4)

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 spasmolytic activity. The test compound dose-dependently (0.05-0.1 mM, n = 4) shifted the Ca^{++} dose-response curves to the right (Fig. 3A), similar to that produced by verapamil (0.3-1.0 μM, n = 4, Fig. 3B), a standard CCB (Bolton, 1979; Godfraind, 1987), thus confirming the CCB activity.

Keeping in mind the widespread utility 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 of rabbit aorta but showed a dose-dependent vasodilator effect (Fig. 4) on high K^+ (75 mM)-induced contractions and control peak responses of NE (1 μM), after pretreating the peaks with each dose of test compound for 1 h, with EC_{50} values of 0.04 mM (0.02-0.08, n = 5) and 0.04 mM (0.03-0.06, n = 5), respectively, thus reiterating the already observed CCB activity. 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 exhibited a dose-dependent negative inotropic effect (Fig. 5) on the ventricular contractility with an EC_{50} value of 0.59 mM (0.04-8.86, n = 5). The reduction in rate of contraction is known to be due to decrease in trans-sarcolemmal Ca^{++} influx (Malecot and Trautwein, 1987) while reduction in force of contraction is the result of inhibition of transmembrane Ca^{++} influx through L-type Ca^{++} channels (Fleckenstein, 1977; Conti *et al*., 1985).

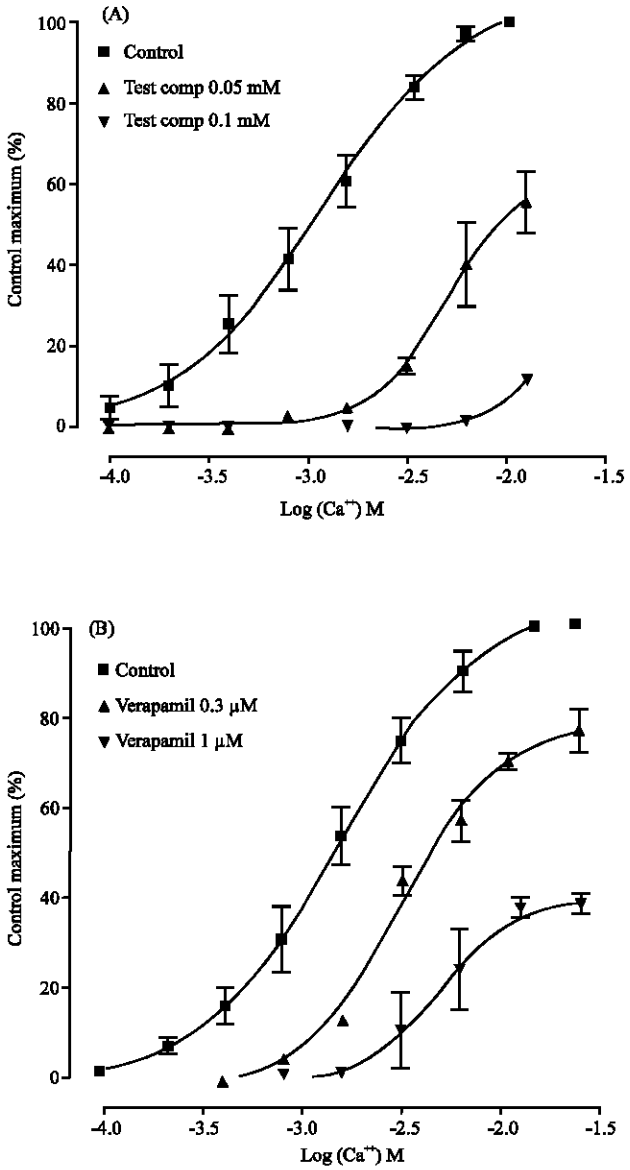

Fig. 3: Dose-response curves showing the inhibitory effect of increasing doses of [A] test compound and (B) verapamil on Ca^{++} concentration-response curves, constructed in a Ca^{++}-free medium, in rabbit jejunum preparations (values shown are mean±SEM, n = 4)

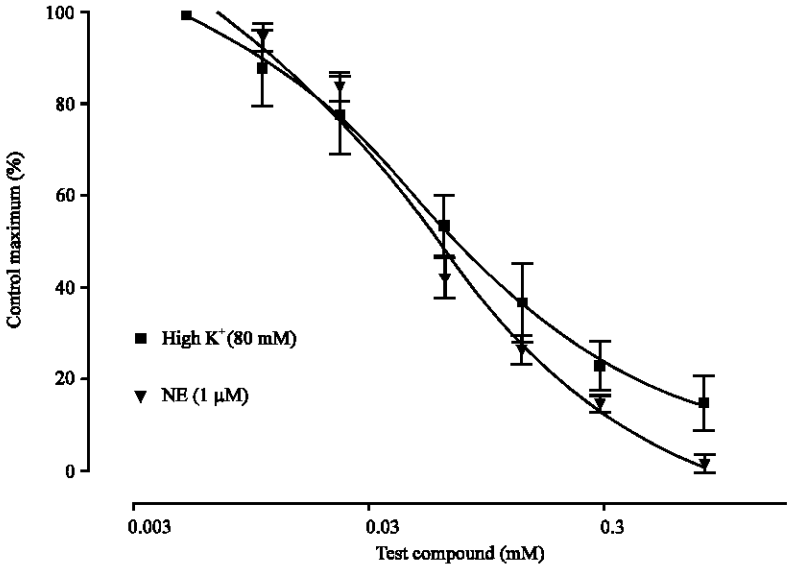

Fig. 4: The inhibitory effect of increasing doses of the test compound on high K^+ (75 mM)-induced contractions and norepinephrine(NE, 1 µM) control peak responses in isolated rabbit aorta (values shown are mean±SEM, n = 5)

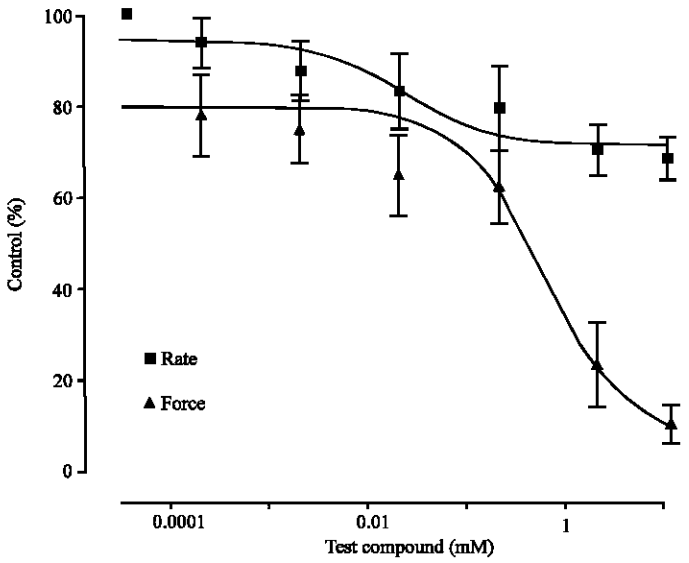

Fig. 5: Dose-response curves showing the inhibitory effect of increasing doses of the test compound on rate and force of ventricular contractions of rabbit whole heart perfused preparation (values shown are mean±SEM, n = 5)

The results showed intestinal spasmolytic, vasodilator and cardio-suppressant activities of the test compound mediated possibly through blockade of voltage- and receptor-operated Ca^{++} channels.

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