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AN *IN VITRO* STUDY OF SELECTIVE CARDIOVASCULAR EFFECTS OF 1-[4'-METHYLPHENACYL]-4-ACETYL -4-PHENYLPIPERIDINIUM BROMIDE

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ABSTRACT: Study of a newly synthesized piperidine compound (1-[4'-methylphenacyl]-4-acetyl-4-phenylpiperidinium bromide) on isolated whole heart of male rabbit, guinea pig ileum and rabbit thoracic aorta were performed for cardiac activity and probable mode of action respectively. Langendorff's technique determined the direct effect on myocardium. Guinea pig ileum and Rabbit aortic rings were employed to delineate the mechanism of action. The test compound reduced the rate and force of contraction markedly. The coronary flow was decreased moderately. The compound failed to induce any spasmogenic or agonistic response in guinea pig ileum. High K^+-induced contraction in rabbit aorta was relaxed dose-dependently. The contractile effect of norepinephrine was inhibited completely. The test compound was found to be cardio-active, devoid of agonistic activity and non-specific calcium antagonist.

KEY WORDS: Chronotropic, Inotropic, Vasoconstricting, Calcium antagonists

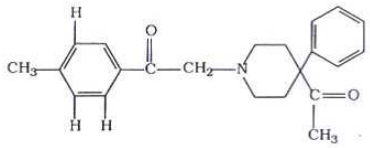
INTRODUCTION

To date, very few piperidine derivatives have been reported which are cardio-active and calcium entry blockers as well. CPU23 is reported as calcium antagonist (Dong *et al.*, 1993). New piperidine analogs were synthesized and reported as calcium antagonist selectively in myocardium. These compounds inhibited force of contraction and heart rate in guinea pig and dogs (Takahara *et al.*, 1999). Bupavacine was reported to exert suppressing effect on $[Ca^{++}]_i$ oscillations in neonatal rat myocaridocytes (McCarlin & Butterworth, 2000). Invitro studies, fentanil, sufentanil and remifentanil were proved to be calcium antagonists and their depressing effect was abolished by 4.0 mM Ca^{++} (Hanouz *et al.*, 2001). 4-arylpiperidine and 4-arylpiperidinols synthesized and showed Na^+ and Ca^{++} channels blocking activity (Annoura *et al.*, 2002). Mepiridine was studied in Langendorff's rat heart & found to exert negative inotropic effects. The authors suggested the inhibition of Ca^{++} current through L-type calcium channel (Zing *et al.*, 2003). But the report lacks the experiment on aorta to confirm the involvement of L-type calcium channel. The effect of *N*-n-butylhaloperidol iodide was studied in rat myocardial ischemia and reperfusion injury. The authors suggested the inhibitory effect was mediated through L-type calcium channels (Huang Zhan *et al.*, 2003).

Current studies report the effects of 1-(4'-methylphenacyl)-4-acetyl-4-phenylpiperidine directly on myocardium and coronary vessels of isolated whole intact heart of rabbit and calcium antagonist activity. Besides cardiac activity the test compound was found to be non-specific calcium antagonist and devoid of agonistic effect.

Chemistry

The chemical structure and molecular formula of 1-(4′-methylphenacyl)–4–acetyl–4-phenyl-piperidinium bromide ($C_{22}H_{26}NO_2.Br$) are as follows. The compound is colorless crystal, melting point is 226-228°C and water-soluble. The detailed chemical synthesis and analgesic activity has been reported (Hameed *et al.*, 1993).



MATERIALS AND METHODS

A) *Drugs and Chemicals.*

The test compound was synthesized and provided by Prof. Dr. Z.S Saify, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, and University of Karachi.

B) *Preparation of Isolated Heart.*

Male healthy rabbits of local breed, weighing 1000 ± 100 g were used to obtain the hearts. The guidelines for ethical use of the experimental animals, laid down by Aga Khan University, were followed.

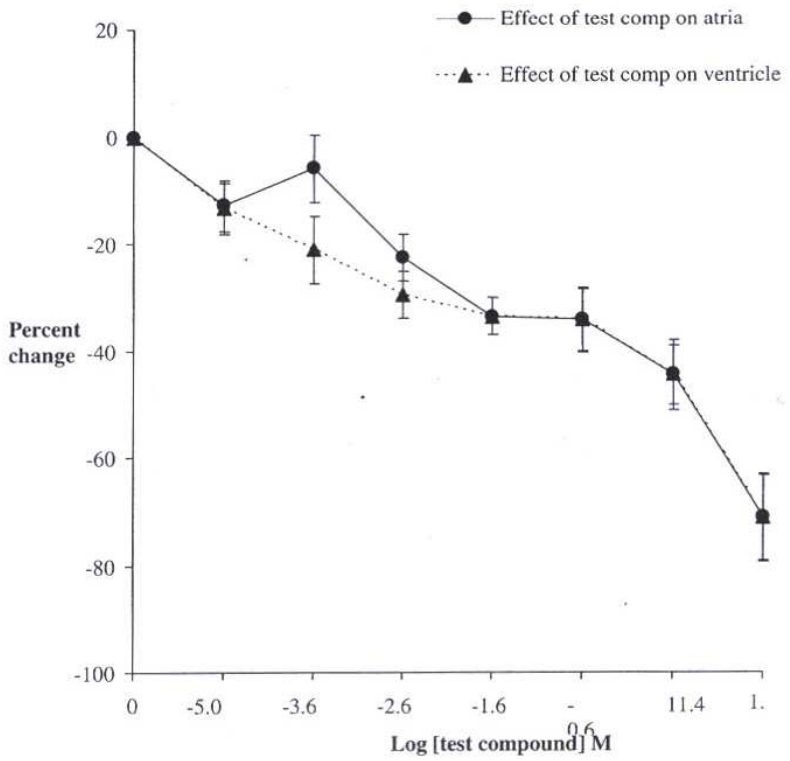
Heparin (5000 I.U) was injected (i.p) one hour prior to isolation of heart. After cervical dislocation, heart was

excised and quickly mounted on the Langendorff apparatus. Krebs-Henseliet solution (composition in g/L: NaCl 6.96, KCl 0.35, KH_2PO_4 0.15, $NaHCO_3$ 2.10, Glucose 0.999, $CaCl_2$ 0.332, $MgSO_4$ 0.29) perfused the heart retrograde, aerated by carbogen (95% O_2 & 5% CO_2) at thermostatically controlled temperature, 37°C. The pH was 7.4. Two different isotonic transducers recorded the atrial and ventricular activities simultaneously and separately. Approximately 60 minutes were allowed to each heart to adapt new environment and to exhibit S.A nodal pattern of the cardiac activity. The heart showing any abnormal pattern was discarded. After taking 10 minutes of control period, the test compound was added in ascending order. For each dose, 10 minutes were allowed to achieve maximum effect of the administered test does. The changes in atrial and ventricular activity were calculated when maximal effect persisted for 5 minutes or more. Total drain was noted by graduated cylinder for the experimental period of 10 minutes after each dose (Staff University of Edinburgh).

C) *Preparation of Guinea pig ileum*

Guinea pig was starved for 24 hr having free access to water. Ileum was dissected out and cut into pieces of 2.0 cm long. Each piece was hung in 10 ml tissue bath having Tyrode's solution (composition in mM: KCl; 2.7, NaCl; 136.9, $MgCl_2$; 1.1, $NaHCO_3$; 11.9, NaH_2PO4; 0.4, $CaCl_2$; 1.8, Glucose; 5.6), aerated with carbogen (95 % O_2 and 5% CO_2) at the maintained temperature of 37°C. Under these conditions, guinea

Fig. 1: Effects of test compound on the rate of atrial and ventricular contraction.Base-line (control) is regarded as 100% in each preparation (shown at 0.0). (n=5)



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pig ileum behaved as quiescent muscle and was considered more suitable to test spasmogenic or agonistic activity of the test compound (Gilani and Aftab, 1992). In guinea pig ileum agonist response was mediated through different receptors inclusivep,opioid receptors predominantly (Kitchen, 1984).

D) *Preparation of Rabbit Aorta*
The descending thoracic aorta was isolated, adjacent tissues was removed and 2-3mm wide rings were cut and mounted in 10 ml tissue bath individually. KrebsHenseliet solution (composition in mM:NaCl 11.50, KCl 4.70, CaCh 2,50, NaHC03 25.0, MgS04.7H2O 1.50, K2H2P04.2H2O 1.20, Glucose 11.0) bathed the tissues, aerated with carbogen (95% 02, 5% CO2) at thermostatically maintained temperature 37°C at the pH. The aortic rings were connected to Grass polygraph model 7H, through force displacement transducer (FT 03). After stabilizing the tissue, 75mM KCl induced the sustained contraction, subsequently, the test compound was added in cumulative dosefashion to obtain concentration dependent response (Van Rossum, 1963).

E) *Contractile Effect of Norepinephrine*
After stabilizing the aortic rings, 111M norepinephrine was added in the bath and this response was taken as control, the effect was completely washed out by repeated washings. Once again, the dose of 111M norepinephrine was added in the presence of the test compound.

E) *Statistical Analysis*
Data were expressed as standard error of means (:t) paired t-test was employed to calculate the level of significance. A level of $p < 0.05$ was considered significant versus control, basal activity is considered control.

RESULTS

A) *Chronotropic Effects*
The results of chronotropic activity are summarized in figure (1). This showed atria and ventricles affected synchronously and produced negative chronotropic effect dose-dependently.

B) *Inotropic Effects*
The inotropic effects are presented in figure (2). Atria and ventricles were affected variably. The most of the doses of test compound in atria induced marked negative inotropic effect except 3rd and 4th doses that decreased the force of contraction only slightly. In the ventricles the negative effect did not progress as doses were increased. The dose of 2x 1O-3M returned the force of contraction at pre-treatment level, while 2 x 1O-2M and 2xlO-'M exhibited the positive inotropic effect considerably. The rest of the doses caused negative inotropic effect statistically significant.

Tracing no 2 & 3 showed significant depressing effect of the doses of 1 and 2 mg on the force and rate of contraction, while (Tracing no. 1) is pre-treatment S.A nodal pattern of atrial and ventricular activity.

C) *Effects on coronary vessels*
The figure (3) presented effects on total drain. The test compound decreased total drain dose-dependently, indicating vasoconstricting effect in the coronary vessels. The constricting effect was moderate and statistically significant.

D) *Effects on Guinea pig ileum*
The test compound was administered in cumulative dose fashion up to 5xlO-3 M. None of the dose could elicit the agonistic response (Tracing no 4).

E) *Effects on Thoracic Aorta*
The sustained contraction induced by high-K+, was relaxed by adding the test compound in cumulative dose-fashion. The effect was initiated at lxl0-6M and completed at 5xlO-4M figure (4).

F) *Inhibition of Contractile Response of Norepinephrine*
The test compound inhibited contractile response of 111M norepinephrine highly significantly (Tracing no.5).

DISCUSSION

The test compound decreased the heart rate dose-dependently and synchronously. Many mechanisms have been postulated for such behavior but reduction in trans-sarcolammel calcium influx (Malecot and Trautwein, 1987). It has been appreciated and well documented. This compound produced variable inotropic effect in atria and ventricles at intermediary lower doses. This effect did not persist any longer. Nevertheless, the force of contraction was decreased significantly and potently at higher doses (Tracing no 2 & 3). The force of contraction in the isolated

perfused heart has also been well established as resulting from inhibition of trans-membrane calcium influx (Fleckenstin, 1977 and Conti *et ai.,* 1985). This is confirmed by the experiments on rabbit aorta. A dose-dependent decrease in the total drain is induced by the test compound. This shows vasoconstriction effect in the coronary vessels. The test compound is not found very potent vasoconstricting agent even at the highest administered dose. It may be assumed that the effect on myocardium is not the result of coronary vessels blockade.

Guinea pig ileum preparation provided a steady base line, which was important to study drug-induced contraction (Kitchen, 1984). The test compound did not exhibit spasmogenic or agonistic activity even at *500fLM* dose; showing the lack of agonistic property of the test compound on the receptors available in guinea pig ileum, notably the *fL* opioid receptors (Kitchen, 1984). Since phenylpiperidines derivatives mimic the action of opioid peptides (North, 1986). Therefore, it can be reasonably thought the test compound lacks the agonistic activity inclusive opioid receptors.

Rabbit thoracic aortic rings were used to determine CaH-antagonistic activity, as proposed by (Karaki *et ai.,* 1986b, Hester, 1985, Fare *et al.,* 1991). The test compound relaxed the high- K+ contracted aorta dosedependently. This supported the notion that test compound was capable of blocking the calcium influx through voltage dependent calcium channels. The addition of IfLM norepinephrine in the presence of test compound exhibited potent and significant inhibitory effect on contractile response. This indicated inhibition of receptor-operated calcium channel (ROCs) as well. Therefore, according to (Karaki, 1986) the test compound was a non-specific Ca $_{H}$ -antagonist. The studies of Dong *et al.,* 1992 corroborated with our results and supported the idea that aorta contains Ltype calcium channels. The test compound which blocked the influx of calcium in aorta might be characterized as calcium antagonist. However, the positive inotropic effect at lower intermediary doses was not significant being abolished at higher doses.

CONCLUSION

The test compound showed marked negative chronotropic and inotropic effect directly acting on myocardium. Coronary vessels were moderately affected. In aorta, the blockade of CaH influx and inhibition of contractile response of norepinephrine by the test compound were supporting evidence to conclude as non-specific calcium antagonist.

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