J. Pharm. Pharmacol. 1999, 51: 953–957
Received March 9, 1999
Accepted March 10, 1999

Overlap in the Pharmacology of L-type Ca^{2+}-channel Blockers and 5-HT_2 Receptor Antagonists in Rat Aorta*

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Abstract

We have previously shown that elimination of buffer Ca^{2+} markedly reduced maximum 5-HT-induced contractions. We have now investigated the effect of L-type Ca^{2+}-channel blockers and 5-HT_2 receptor antagonists on 5-HT- and K^+-induced contractions in rat aorta to explore the possibility of a relationship between blockade of L-type Ca^{2+} channels and 5-HT_2 receptor antagonism.

Sodium nitroprusside, felodipine, nifedipine, diltiazem, cinnarizine, verapamil, ritanserin, cyproheptadine, ketanserin and mianserin inhibited 5-HT-induced contractions of rat aorta with mean IC50 values (concentration (M) resulting in 50% inhibition) of $2{\cdot}2 \times 10^{-11}$, $6{\cdot}6 \times 10^{-11}$, $1{\cdot}5 \times 10^{-9}$, $1{\cdot}7 \times 10^{-9}$, $3{\cdot}2 \times 10^{-7}$, $5{\cdot}4 \times 10^{-7}$, $9{\cdot}7 \times 10^{-10}$, $1{\cdot}9 \times 10^{-8}$, $5{\cdot}0 \times 10^{-7}$ and $6{\cdot}4 \times 10^{-7}$, respectively. The same compounds antagonized K^+-induced rat aortic contractions with the rank order of potency (mean IC50, M): felodipine ($7{\cdot}0 \times 10^{-11}$) > nifedipine ($4{\cdot}8 \times 10^{-9}$) > sodium nitroprusside ($4{\cdot}1 \times 10^{-8}$) > verapamil ($5{\cdot}5 \times 10^{-8}$) > cyproheptadine ($6{\cdot}2 \times 10^{-8}$) > diltiazem ($4{\cdot}1 \times 10^{-7}$) > cinnarizine ($1{\cdot}3 \times 10^{-6}$) > ritansarin ($1{\cdot}8 \times 10^{-6}$) > ketanserin ($9{\cdot}0 \times 10^{-6}$) > mianserin ($2{\cdot}0 \times 10^{-5}$).

These data are indicative of a highly significant correlation ($r = 0{\cdot}81$, $P = 0{\cdot}03$) between potency against 5-HT-induced contraction and that against contractile response to K^+ depolarization, and suggest overlap of the pharmacology of L-type Ca^{2+}-channel blockers and 5-HT_2 receptor antagonists in rat aorta.

The origin of this work was the observation (Okoro et al 1995) that exposure of arterial smooth muscle preparations from rat to the L-type Ca^{2+}-channel blocker verapamil prevented the subsequent antagonistic action of ketanserin (a 5-HT_2 receptor antagonist) against 5-HT-induced contractions. More recently Okoro & Marwood (1997a) observed that 5-HT_2 receptor antagonists have activity at a site in Ca^{2+} channels that is involved in the activation of L-type Ca^{2+} channels. Because these observations are consistent with the possibility of overlap of the pharmacology of L-type Ca^{2+}-channel blockers and 5-HT_2 receptor antagonists in arterial smooth muscle, we studied the separate effects of a series of compounds (L-type Ca^{2+}-channel blockers or 5-HT_2 receptor antagonists) on rat aortic contractions evoked by 5-HT and K^+ to explore the possibility of a relationship between blockade of L-type Ca^{2+} channels and 5-HT_2 receptor antagonism. The main aim of the study was to observe, through effects on contraction amplitude, any link between inhibition of 5-HT_2 receptor and L-type Ca^{2+} channels in arterial smooth muscle. This report therefore supplements data which have appeared in the papers cited above.

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*This report is dedicated to the memory of John Shaw, MD, FRACP, late Professor of Clinical Pharmacology, University of Sydney, Australia.

Materials and Methods

Basis of the experimental design

The study was performed on isolated rat aorta because it contains Ca^{2+} channels (Karaki et al 1984; Okoro & Marwood 1997a) and 5-HT_2 receptors (Okoro et al 1995), and also for reasons that will be clarified below.

Drugs used

The drugs used were 5-hydroxytryptamine creatinine sulphate, diltiazem and cinnarizine from Sigma (St Louis, MO), prazosin HCl (Pfizer,

Australia), sodium nitroprusside (Roche Products, Australia), nifedepine (Bayer, Australia), felodipine (Astra Pharm, Australia) and verapamil. Drug solutions were freshly prepared daily; all drugs were dissolved in distilled water except prazosin, nifedipine and cinnarizine which were initially dissolved in dimethylsulphoxide (DMSO) and then diluted with distilled water. Control responses were measured in the presence of DMSO where appropriate. The highest concentration of DMSO in the physiological salt solution never exceeded 0·01% (v/v) and no measurable effects of DMSO on aortic contractions were observed—as reported previously (Okoro 1993; Okoro et al 1995).

Experimental procedures

The procedure and solutions used have been described previously (Okoro et al 1995; Okoro & Marwood, 1997a, b). Essentially, thoracic aorta of male and female Sprague–Dawley rats, 250–450 g, were cut into helical strips and positioned vertically in a 10-mL organ bath containing physiological salt solution thermostatically regulated at 37°C and continually bubbled with 5% carbogen. Resting tension was equivalent to a load of 0·5 g and the equilibration time was 2 h. Isometric contractions were recorded with a Narco-Biosystems model F-60 force transducer connected to a chart recorder (Graphtec MK VII).

Contractions evoked by 5-HT were measured as increases in baseline tension and expressed as a percentage of the maximum increase in tension induced by 10^{-4} M 5-HT (which produced the maximum response). Dose–response curves to 5-HT were established by cumulatively increasing the concentration of 5-HT in the organ bath until maximum response was observed. After construction of control 5-HT dose–response curves, tissues were perfused for 45 min with physiological salt solution containing an antagonist drug, before re-exposure to 5-HT. The procedure was repeated with increasing concentrations of antagonist drugs. It has been established (Okoro 1993; Okoro et al 1995) that the rat aortic strip is stable to repeated exposure to 5-HT for at least 7 h. The data on K^+-induced contractions were generated as reported previously (Okoro & Marwood 1997a).

Results are presented as group mean ± s.e.m. unless stated otherwise. The significance of changes in contractile response was evaluated by use of *t*-test statistics and differences were considered to be significant when $P < 0.05$. As a measure of antagonist potency the drug concentration that resulted in 50% inhibition of the contraction induced by 10^{-4} M 5-HT (IC50) was derived by simple reverse linear regression of antagonist doses on responses to 10^{-4} M 5-HT or 60 mM K^+ (Okoro et al 1995; Jolayemi & Okoro 1996; Okoro & Marwood 1997a). The strength of association between the antagonism of 5-HT-induced contractions and blockade of contractions evoked by K^+ was assessed by regression analysis after logarithmic transformation of IC50 data.

Results

Effect of prazosin on responses to 5-HT

This drug did not reduce 5-HT-induced contractions and at 10^{-5} M, augmented aortic responses to lower concentrations of 5-HT (Figure 1).

Effects of selected antagonist drugs on aortic responses to 5-HT

Nifedipine (10^{-9}, 10^{-8} and 10^{-7} M) dose-dependently reduced maximum aortic responses to 5-HT; the mean IC50 was 1.5×10^{-9} M. Felodipine (10^{-11}, 3×10^{-11}, 10^{-10} and 10^{-9} M) dose-dependently antagonized 5-HT-induced aortic contractions and significantly reduced the maximum responses to 5-HT to 69·7 ± 5·8, 54·3 ± 5·8, 38·1 ± 7·1 and 13·7 ± 4·0%, respectively, of the control value (100%). These results suggest that felodipine was approximately 22 times more potent than nifedipine against 5-HT-induced contractions (Table 1). Diltiazem (10^{-9}, 10^{-8} and 10^{-7} M) dose-dependently antagonized 5-HT and significantly reduced the maximum contractile responses to 5-HT (10^{-4} M) to 78·2 ± 6·2, 59·8 ± 5·4 and 43·2 ± 4·9%, respectively, of the control value. Thus, diltiazem and nifedipine had comparable

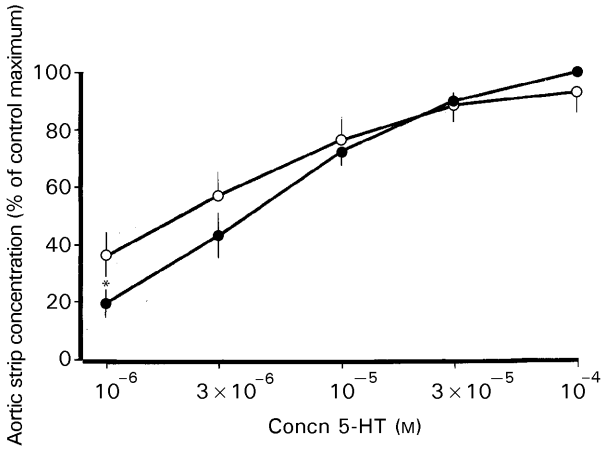

Figure 1. The effect of prazosin (○; 10^{-5} M) on aortic responses to 5-HT. Contraction is expressed as a percentage of the control response (●; zero prazosin) to 10^{-4} M 5-HT. Each bar represents the mean ± s.e.m. of results from six different determinations. * $P < 0.05$ compared with control.

Table 1. Inhibitory activity of antagonist drugs against contractions of rat aortic strips evoked by 5-HT or K^+.

Antagonist	IC50 5-HT (M)	IC50 K^+ (M)
Sodium nitroprusside	$2{\cdot}2 \pm 0{\cdot}2 \times 10^{-11}$	$4{\cdot}1 \pm 0{\cdot}4 \times 10^{-8}$
Felodipine	$6{\cdot}6 \pm 0{\cdot}6 \times 10^{-11}$	$7{\cdot}0 \pm 0{\cdot}5 \times 10^{-11}$
Nifedipine	$1{\cdot}5 \pm 0{\cdot}2 \times 10^{-9}$	$4{\cdot}8 \pm 0{\cdot}4 \times 10^{-9}$
Diltiazem	$1{\cdot}7 \pm 0{\cdot}1 \times 10^{-9}$	$4{\cdot}1 \pm 0{\cdot}4 \times 10^{-7}$
Cinnarizine	$3{\cdot}2 \pm 0{\cdot}1 \times 10^{-7}$	$1{\cdot}3 \pm 0{\cdot}1 \times 10^{-6}$
Verapamil	$5{\cdot}4 \pm 0{\cdot}1 \times 10^{-7}$	$5{\cdot}5 \pm 0{\cdot}3 \times 10^{-8}$
Ritanserin	$9{\cdot}7 \pm 0{\cdot}8 \times 10^{-10}$	$1{\cdot}8 \pm 0{\cdot}1 \times 10^{-6}$
Cyproheptadine	$1{\cdot}9 \pm 0{\cdot}5 \times 10^{-8}$	$6{\cdot}2 \pm 0{\cdot}7 \times 10^{-8}$
Ketanserin	$5{\cdot}0 \pm 0{\cdot}2 \times 10^{-7}$	$9{\cdot}0 \pm 1{\cdot}1 \times 10^{-6}$
Mianserin	$6{\cdot}4 \pm 0{\cdot}8 \times 10^{-7}$	$2{\cdot}0 \pm 0{\cdot}2 \times 10^{-5}$

The IC50 is the concentration of drug resulting in 50% inhibition of the contraction induced by 10^{-4} M 5-HT.

potency against aortic responses to 5-HT (Table 1). Cinnarizine (10^{-7}, 10^{-6} and 10^{-5} M) dose-dependently suppressed aortic responses to 5-HT and significantly reduced maximum responses to 5-HT to $79{\cdot}3 \pm 4{\cdot}9$, $44{\cdot}0 \pm 8{\cdot}1$ and $11{\cdot}7 \pm 1{\cdot}7$%, respectively, of the control value (100%). These data indicate that cinnarizine was (approx.) 46 times less potent than nifedipine against 5-HT-induced aortic contractions (Table 1).

We have shown previously (Okoro et al 1995) that at concentrations of 10^{-7}, 3×10^{-7} and 10^{-6} M, verapamil dose-dependently antagonized 5-HT-induced rat aortic contractions and significantly reduced maximum responses to $76{\cdot}5 \pm 2{\cdot}0$, $55{\cdot}0 \pm 5{\cdot}5$ and $31{\cdot}0 \pm 3{\cdot}5$%, respectively, of the control value (100%). By use of the methods described herein an IC50 value of $5{\cdot}4 \pm 0{\cdot}1 \times 10^{-7}$ M was derived.

Sodium nitroprusside (10^{-11}, 3×10^{-11}, 10^{-10} M) dose-dependently reduced aortic responses to 5-HT and significantly suppressed maximum responses to 10^{-4} M 5-HT. The data (see Table 1) indicate that this drug was the most potent, being (approx.) 66 times more active than nifedipine against aortic responses to 5-HT.

Effect of sodium nitroprusside on rat aortic responses to K^+

Sodium nitroprusside (10^{-9}, 10^{-8} and 10^{-7} M) dose-dependently inhibited rat aortic responses to K^+ and reduced maximum K^+-induced contractions to $83{\cdot}7 \pm 4{\cdot}2$, $50{\cdot}7 \pm 3{\cdot}4$ and $25{\cdot}3 \pm 3{\cdot}0$%, respectively, of control maximum; the mean IC50 was $4{\cdot}1 \times 10^{-8}$ M.

Discussion

In discussing the possible implications of these data, several issues must be considered. Firstly, the experiment summarized in Figure 1 was undertaken because of convincing evidence (Marwood & Stokes 1983; Marwood 1988) that activation of α-adrenoceptors can contribute to 5-HT-induced contractions in isolated arterial preparations of rat. However, the results (Figure 1) show that even at concentrations (10^{-5} M) 1000 times greater than those that normally block α-adrenoceptors (Adachi & Shoji 1986; Leysen et al 1988) prazosin did not reduce 5-HT-induced contractions. This observation is consistent with previous data (Low et al 1994) which indicate lack of involvement of α-adrenergic activation in rat aortic responses to 5-HT. Therefore, the 5-HT_2 receptor in this tissue seems to be pharmacologically distinct from α-adrenoceptors (Figure 1) and it was possible to investigate the 5-HT_2 receptor relatively separately, and its link with Ca^{2+} influx, without interference from α-adrenoceptors (Marwood 1988).

Secondly, rat aortic contractions evoked by 5-HT depend on Ca^{2+} influx (Okoro & Marwood 1997b) and the current data indicate that the resulting contractions are sensitive to blockade by a series of L-type Ca^{2+}-channel antagonists as documented previously (Gouw et al 1989; Iwanov & Mould 1991). The current IC50 values (Table 1) are, however, different from those reported by the investigators cited above. It should be noted that our method of assessing antagonist potency against 5-HT-induced contractions was similar to those of Gouw et al (1989) but different from those of Iwanov & Mould (1991). Further, many of these studies were undertaken on aorta derived from different rat strains, and tissue contact time with antagonist drugs was brief, often less than 20 min, compared with 45 min or more in this study. These experimental differences, particularly the longer incubation time, might account for the higher potency against 5-HT-induced contractions observed in the current study.

The comparative inhibitory activities of antagonist drugs examined are shown in Table 1. The IC50 values (Table 1) were derived from this study and taken from Okoro et al (1995) and Okoro & Marwood (1997a, b). Strikingly, contractions evoked by 5-HT were as sensitive to the L-type Ca^{2+}-channel blockers as those induced by K^+ depolarization, and occasionally even more sensitive. This finding is at variance with previous data (Mulhern & Docherty 1989; Polster et al 1990) which indicate lower sensitivity of 5-HT-induced contractions to L-type Ca^{2+}-channel blockers when compared with those evoked by K^+. These observations prompted the studies with sodium nitroprusside, because the literature (Karaki et al 1984; Chang et al 1993; Low et al 1994) indicates that

Ca^{2+} uptake and the resulting contractions evoked by receptor agonists are preferentially inhibited by sodium nitroprusside when compared with K^+-induced influx through L-type Ca^{2+} channels, thus suggesting that this compound might have sufficient selectivity to discriminate between Ca^{2+} uptake through receptor-operated Ca^{2+} channels and those through L-type Ca^{2+} channels. However, the observation (Table 1) that K^+-induced contractions of rat aorta are sensitive to sodium nitroprusside is therefore, at variance with findings from rabbit and guinea-pig aortae where K^+-induced contractions are resistant to sodium nitroprusside even at doses as high as 10^{-5} M (Karaki et al 1985; Chang et al 1993; Low et al 1994). This might indicate that channels through which 5-HT and K^+ mobilize external Ca^{2+} for contractions are functionally linked in rat aorta.

In this regard, it is important to note that a superficial comparison of the potencies listed in Table 1 did not reveal any uniformity between potency against 5-HT-induced contractions and potency against K^+-induced contractions, except for felodipine, nifedipine and cyproheptadine, for which potency against contractions evoked by 5-HT and K^+ were comparable. In contrast, the 5-HT_2 receptor antagonists seemed at least 100 times more active against aortic responses to 5-HT compared with those induced by K^+. Because the heterogeneity in the chemical structures of the drugs is such that no structure–activity relationship was discernible, and to test more stringently for any possible correlation between the 5-HT and K^+ antagonist properties of the compounds, regression analysis was performed using the IC50 values for the ten compounds. This treatment revealed highly significant ($r = 0{\cdot}81$, $P = 0{\cdot}03$) positive correlation between potencies at 5-HT_2-receptors and at L-type Ca^{2+} channels. The regression analysis yielded the equation $Y = 0{\cdot}73X + 3{\cdot}7$ where 0·73 is the slope of the regression line and 3·7 the intercept on the Y axis. When the same analysis was repeated using only the IC50 data for the classic L-type Ca^{2+}-channel blockers and sodium nitroprusside, the correlation coefficient (r) increased ($r = 0{\cdot}92$, $P = 0{\cdot}004$) and the regression equation changed to $Y = 1{\cdot}01X + 0{\cdot}06$.

This highly significant correlation between blockade of L-type Ca^{2+} channels (activated by K^+) and antagonism of 5-HT-induced responses by the ten compounds studied reinforces the proposition that 5-HT and K^+ might activate Ca^{2+} uptake into rat aortic smooth muscle by a common mechanism. Further, this systematic relationship could indicate that a change in the activity of L-type Ca^{2+} channels is accompanied by a proportional change in the effect of 5-HT_2 receptor functioning.

In addition to these considerations, several other observations are germane. Firstly, at concentrations similar to those used to evoke the contractions measured in this study 5-HT has been reported (Doggrell et al 1989) to depolarize the membrane potential of several types of arterial smooth muscle preparation, including rat aorta, thus indicating that 5-HT might promote Ca^{2+} uptake through L-type Ca^{2+} channels. Secondly, at the concentrations used, none of the L-type Ca^{2+}-channel blockers has any measurable activity at the 5-HT_2 receptor (Norris & Bradford 1985; Adachi & Shoji 1986). Thirdly, the nature (non-surmountable) of antagonism against 5-HT-induced contractions is at variance with simple antagonism, thus suggesting a more complex interaction beyond simple receptor binding.

In conclusion, these findings suggest a link in the pharmacology of L-type Ca^{2+}-channel blockers and 5-HT_2 receptor antagonists.

Acknowledgements

The author thanks Professor E. T. Jolayemi of the Department of Statistics, University of Ilorin, Nigeria, for his assistance in analysing the data. The helpful comments of Professor Ebun Davies, University of Ilorin, on the draft manuscript are gratefully acknowledged.

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