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EFFECTS OF CYPROHEPTADINE, RITANSERIN AND Ca2+ ON RAT AORTIC REACTIVITY TO 5-HYDROXYTRYPTAMINE

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ABSTRACT: A study was undertaken to determine whether the similarity in the profile of action of Ketanserin (a $5HT_2$ receptor antagonist and a series of L-type Ca^{2+} channel antagonists previously reported (1,2) is shared by other chemically unrelated $5HT_2$ receptor antagonists. Cyproheptadine and ritanserin in nanomolar concentrations potently suppressed 5HT-induced contractions of rat aortic strip non-surmountably. Increasing buffer Ca^{2+} concentrations from 2.0 mM to 5.0 mM in the continued presence of the same concentrations of each drug attenuated the expected rise in the inhibitory activity of these compounds over time. These findings are strikingly similar to those previously noted with ketanserin. Together with previous data (1-4), the present observations suggest overlap in the pharmacology of $5HT_2$ receptor antagonists and L-type Ca^{2+} channel antagonists.

Key Words: 5-Hydroxytryptamine; Cyproheptadine; Ritanserin; Ketanserin; Ca^{2+} channels; Rat aorta.

INTRODUCTION

Our previous report (1) showed that blockade of 5HT-induced contractions of isolated arterial preparations of rat by ketanserin (a $5HT_2$ receptor antagonist) and the L-type Ca^{2+} channel antagonist verapamil is attenuated by increasing extracellular Ca^{2+}. More recently, we have demonstrated (2,3) that blockade of rat aortic responses to 5HT by a wide range of L-type Ca^{2+} channel antagonists is also diminished by increasing buffer Ca^{2+} concentrations.

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Because these observations suggest some similarity in the action of ketanserin and those of L-type Ca^{2+} channel antagonists, the present study was undertaken to determine whether this similarity extends to other $5HT_2$ receptor antagonists. This communication compliments our previously published data (1-4).

MATERIALS AND METHODS

The experimental procedures and solutions used have been previously described (1 - 4). Essentially, spiral strips of thoracic aorta from Sprague-Dawley rats

were prepared and suspended in a 10 ml double jacketed organ bath containing Physiologic Salt Solution (PSS). The PSS had the following composition (in millimolar): NaCl 118; KCl 4.7, KH_2PO_4 1.8; $MgSO_4.7H_2O$ 0.56; $NaHCO_3$ 25; Glucose 11.1; $CaCl_2$ 2.0 and was continuously bubbled with 5% Carbogen and thermostatically maintained at 37^oC. Tissues were subjected to a resting tension of 0.5g.

A 2 hour equilibration time was allowed for tissue recovery during which time PSS was changed every 15 minutes and baseline tension readjusted. Dose response curves were established by cumulatively increasing 5HT concentrations in the organ bath until maximum response was attained. In experiments with antagonists, a 45 minutes incubation in drugs was allowed before constructing the dose response curve. Thereafter, tissues were repeatedly washed with PSS containing the same drug concentration and PSS containing 5.0 mM Ca^{2+} until baseline was re-established (approximately 30 minutes). Only one antagonist concentration and two different Ca^{2+} concentrations were tested in each preparation as indicated previously (1,3).

Contractile responses to 5HT were recorded isometrically as increases in baseline tension on a Graphtec MK VII chart recorder connected to a force transducer (Narco Biosystems Model F-60).

The drugs used were ritanserin (Janssen-Cilag, Lane Cove, NSW, Australia), Cyproheptadine HCl (Sigma Chemicals, St. Louis, MO, USA) and 5-hydroxytryptamine creatinine sulphate (Sigma Chemicals, St. Louis, MO, USA).

Statistical Analysis

The results are expressed as group mean ± SEM calculated from pooled results of six or more arteries each from a different animal. The significance of changes in contractile responses were evaluated using Student's t-test and the level of significance was fixed at $P < 0.05$ and indicated in figures as asterisks.

RESULTS

Cyproheptadine (3 x 10^{-8}M): In the presence of 2.0 mM Ca^{2+} cyproheptadine significantly attenuated contractile responses to all concentrations of 5HT (Fig. 1). Elevating the buffer Ca^{2+} concentration to 5.0 mM in the continued presence of 3 x 10^{-8}M cyproheptadine did not reduce aortic responses to 5HT beyond that observed in 2.0 mM Ca^{2+}. Indeed, the 5HT dose response curve was shifted to the left of the preceding one in a non-parallel fashion, i.e. responses to the lowest concentration of 5HT was unaffected by the increased Ca^{2+} concentration (Fig. 1).

Ritanserin (3 x 10^{-10}M): As with cyproheptadine, increasing the buffer Ca2+ concentration to 5.0 mM from 2.0 mM in the continued presence of 3 x 10^{-10}M ritanserin had no further inhibitory effect on responses to 5HT beyond those observed with 2.0 mM Ca^{2+} and 3 x 10^{-10}M ritanserin (Fig. 2). However, the 5HT dose-response curve in the presence of 5.0 mM Ca^{2+} was non-significantly shifted to the left of the preceding one except at the lower end of the dose-response curve where both curves converged (Fig. 2). In particular, maximum responses to 5HT in the presence of 3 x 10^{-10}M ritanserin and 5.0 mM Ca^{2+} was 109% of that in the presence of the same drug concentration and 2.0 mM Ca^{2+}. Similar magnitude of change was also observed with cyproheptadine.

DISCUSSION

The present observation is strikingly similar to our previous findings (1-3) with ketanserin (another $5HT_2$ receptor antagonist) and a diverse range of L-type Ca^{2+} channel antagonist, i.e. increasing external Ca^{2+} concentration from 2.0 mM to 5.0 mM attenuated the expected increase in potency of these compounds against 5HT-induced concentrations over

time. This could suggest that modulation of Ca^{2+} uptake may be involved in the antagonist effect of these drugs.

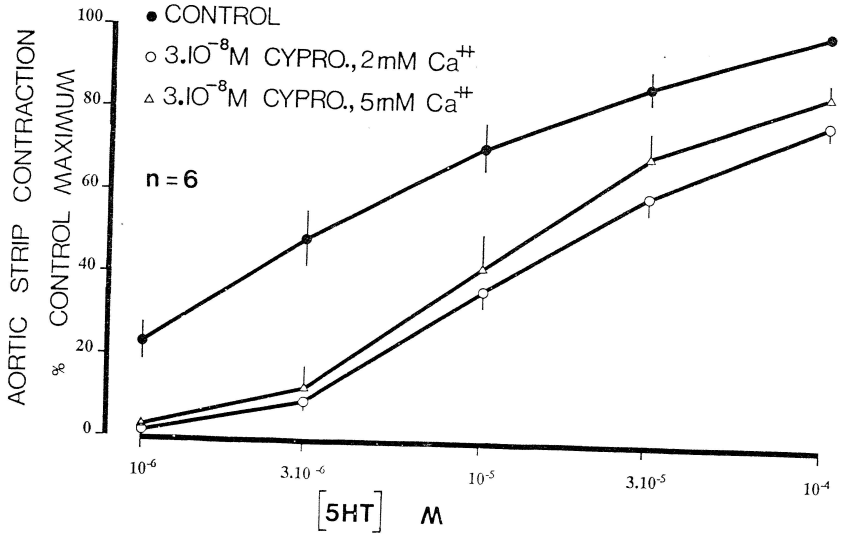

Fig. 1: The effect of raising extracellular [Ca^{2+}] on the antagonism by cyproheptadine of aortic responses to 5HT.

Contraction is expressed as a percentage of the control (zero cyproheptadine and 2.0 mM Ca^{2+}) maximum. Successive dose-response curves were separated in time by at least 60 minutes. Each point on each curve represents the mean ± SEM of six different experiments.

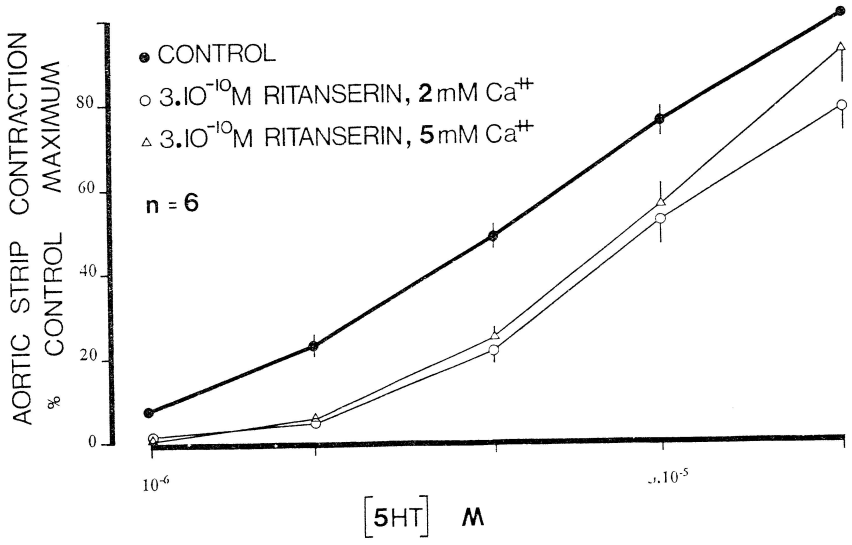

Fig. 2: The effect of raising extracellular [Ca^{2+}] on the antagonism by ritanserin of aortic responses to 5HT.

Contraction is expressed as a percentage of the control (zero ritanserin and 2.0 mM Ca^{2+}) maximum. Successive dose-response curves were separated in time by at least 60 minutes. Each point on each curve represents the mean ± SEM of six different experiments.

Although there is abundant evidence (1,5-7) showing that 5HT induced contractions of rat aorta results largely from Ca^{2+} influx, it is not clear that this is exclusively through $5HT_2$ receptor linked Ca^{2+} channels (8-11). Therefore, it is not impossible that these antagonists may have an action beyond direct activity at the $5HT_2$ receptor as documented recently (4). Data from electrophysiologic studies (12-15) have indicated that at concentrations similar to those used in this study, 5HT can depolarize the membrane potential of several arterial preparations including rat aorta. According to these reports, Ca^{2+} uptake through L-type Ca^{2+} channels may be involved in rat aortic response to 5HT as suggested previously (10). This conclusion is reinforced by our earlier observations (1-3) that 5HT-induced contraction of rat aorta is extremely sensitive to inhibition by a series of L-type Ca^{2+} antagonists and the $5HT_2$ receptor antagonist ketanserin. Incidentally, this latter action is also influenced by external Ca^{2+}.

We cannot determine, from the present study, whether the similarity between these antagonists of $5HT_2$ receptor and L-type Ca^{2+} channels antagonists represent an interplay between both receptor types. However, our recent observation (4) in the same tissue that a series of L-type Ca^{2+} channel antagonists and $5HT_2$ receptor antagonists can interfere with Ca^{2+} uptake through L-type Ca^{2+} channels is consistent with this possibility. In conclusion, therefore, the present results extend our earlier findings with Ketanserin and suggest some overlap in the pharmacology of antagonists of $5HT_2$ receptor and L-type Ca^{2+} channel antagonists.

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