LETTER

Verapamil Interacts Stereoselectively with the Muscarinic Receptor

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Received: November 8, 1984; accepted: November 20, 1984.

Verapamil, a calcium channel blocker, is a highly lipophilic compound that interacts with many known pharmacologic receptors (1, 2). Many of these interactions occur at higher concentrations than those necessary to elicit calcium channel blocking effects and as such may not be relevant under normal therapeutic conditions. Such interactions may involve competitive, 3-point binding processes or noncompetitive lipophilic membrane interactions. An example is the interaction of verapamil with the muscarinic receptor (2). Using *d*- and *dl*-verapamil, we studied the nature of this interaction.

Racemic verapamil was supplied by Knoll Pharmaceuticals (Whippany, New Jersey). *d*-Verapamil was synthesized (3). Since attempts to synthesize optically pure *l*-verapamil were unsuccessful, we compared *d*- and *dl*-verapamil. Male Sprague-Dawley rats were sacrificed by cervical dislocation. The hearts were removed and placed in ice-cold buffer (20 mM HEPES, 10 mM NaCl, 100 mM $MgCl_2$, pH 7.4). The tissue was homogenized and centrifuged at 48,000 x g for 10 min. The supernatant was discarded and the pellet washed once. Aliquots of 0.5 ml (0.85 mg protein/ml) of the crude membrane preparations were incubated with 0.25 nM ^{3}H-*N*-methylscopolamine (^{3}H-NMS; 84.8 Ci/mmole; New England Nuclear, Boston, Massachusetts) in triplicate together with various concentrations of *d*- or *dl*-verapamil. Incubations were carried out at 25 °C for 20 min. Bound ^{3}H-NMS was separated from unbound radioligand by rapid centrifugation (5 min at 15,000 x g). The pellet was solubilized with Protosol (New England Nuclear). Radioactivity was determined by scintillation counting. Nonspecific binding was defined as ^{3}H-NMS binding that occurred in the presence of 10^{-5} M 3-quinuclidinyl benzilate (USPC Inc., Rockville, Maryland). Five paired competition curves with *d*- and *dl*-verapamil were carried out. From these curves the Ki's were calculated (4) and compared using a Student's paired t-test.

Fig. 1 shows representative competition curves for *d*- and *dl*-verapamil. *d*-Verapamil was somewhat weaker than *dl*-verapamil in antagonizing ^{3}H-NMS binding. Similar results were seen in each of the 5 pairs of competition curves (Fig. 2). The mean ± SD Ki for *d*-verapamil was 53.1 ±18.8 μM and 21.0 ±4.1 μM for *dl*-verapamil ($p < 0.05$).

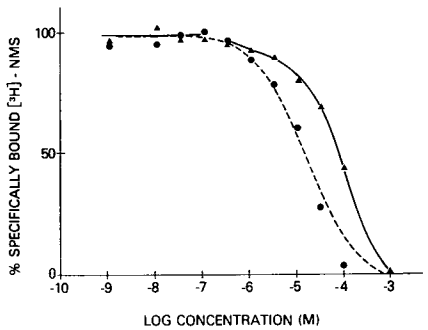

Fig. 1 Displacement of ^{3}H-NMS binding by *dl*-verapamil (●) and *d*-verapamil (▲) in rat myocardial membrane particulates. Each data point represents a mean of triplicate determinations. Experiments were carried out at 25°C in the same myocardial preparation.

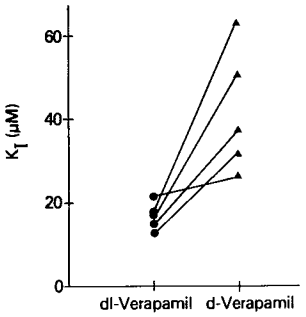

Fig. 2 Inhibition constant (Ki) of *d*- and *dl*-verapamil at the muscarinic receptor. Data were obtained as described in the text from 5 paired experiments.

Our findings demonstrate that the interaction of verapamil with the muscarinic receptor is stereoselective. On the assumption that *d*- and *l*-enantiomer binding is additive, a Ki of approximately 10 μM for *l*-verapamil would be predicted from these data suggesting the degree of stereoselectivity between enantiomers is approximately five-fold. This difference in potency is considerably less than the 100-fold difference in potency between enantiomers in eliciting calcium channel blocking effects in isolated cardiac tissue (5). Furthermore, the plasma concentrations of *l*-verapamil required to produce calcium channel blocking effects *in vivo* are approximately 10 to 100 nM which are considerably lower than the concentrations observed in this study to interact with the muscarinic receptor. These data suggest that antimuscarinic effects will not occur at therapeutic plasma concentrations.

The observed stereoselectivity of verapamil in binding to the muscarinic receptor is in contrast to the findings of Jim at al. who observed that D600 (methoxyverapamil) interacts non-stereoselectively with the muscarinic receptor (1). The observed Ki of both enantiomers of D600 was 7 to 8 μM which is similar to the Ki of *l*-verapamil calculated in this study. Since D600 is more lipophilic than verapamil, it is possible that its interaction may involve primarily lipophilic membrane effects and as such may not be stereoselective. Alternatively, the degree of stereoselectivity of D600 may be lower than that of verapamil at the muscarinic receptor.

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Since these investigators did not carry out paired studies, it is possible that variability in their data may have obscured stereoselective differences in binding (1). Standard deviations were not reported (1).

In conclusion, our data reaffirm that verapamil interacts with other membrane receptors and demonstrate that these interactions, although occurring at high concentrations, may involve 3-point competitive, stereoselective binding processes.

References

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