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# Phosphodiesterase inhibition by papaverine and structurally related compounds*

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Cyclic 3′,5′-nucleotide phosphodiesterase(s) (PD) catalyze the hydrolysis of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) and other cyclic 3′,5′-nucleotides to their respective 5′-nucleoside monophosphates. Following its discovery by Rall and Sutherland,[1] numerous agents have been found which inhibit the PD-promoted hydrolysis of cyclic AMP.[2] Papaverine, initially observed by, Pöch *et al.*[3] to be a potent inhibitor of rat coronary vessel PD, has been reported to exert a similar effect upon this enzyme activity from several different tissues in a number of species.[4–7] Recently structure–activity relationship studies in regard to modifiers of this enzyme activity have appeared in the literature.[8,9] This report describes the investigation of several compounds structurally related to papaverine as inhibitors of a partially purified PD from beef heart. Analysis of the data proved useful in identifying those structural features of papaverine-like compounds which apparently are necessary for inhibition.

Cyclic nucleotide PD was partially purified from beef heart by the procedure of Butcher and Sutherland.[10] The activity of the PD preparation was approximately 4·8 μmoles of cyclic AMP hydrolyzed/mg of protein/hr with saturating amounts of cyclic AMP ($5 \times 10^{-4}$ M) as substrate. Initial velocity measurements were conducted by determining the rate of $^3$H-5′-AMP generation from $^3$H-cyclic AMP according to a procedure described previously.[11] All analyses were carried out in duplicate for 15

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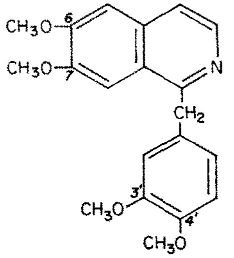
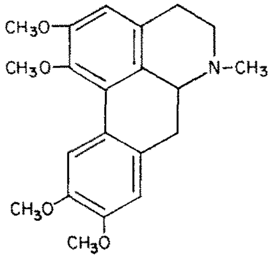
min at 30° in a medium containing 50 mM Tris-HCl, pH 8·0, 2 mM $MgCl_2$, 0·25 mM EDTA and 0·02% bovine serum albumin. A range of substrate concentrations ($3 \times 10^{-7}$ to $3 \times 10^{-5}$ M) approximating the tissue levels of cyclic AMP were employed in these experiments. Under these conditions the apparent $K_m$ value was $10^{-5}$ M; however, higher apparent $K_m$ values are detectable when larger concentrations of substrate are employed.[10] $K_i$ values for the compounds were determined by the method of Dixon[12] or calculated from the apparent kinetic constants ($K_m$ and $V_{max}$) derived from the regression analysis described by Cleland.[13] Both methods produced essentially identical values.

TABLE 1. INHIBITORY POTENCY OF PAPAVERINE AND STRUCTURALLY RELATED COMPOUNDS*



Papaverine



Glaucine

| Compounds | $K_i \times 10^{-6}$ M† | Activity relative to papaverine (%) | Type of inhibition |
|---|---|---|---|
| Papaverine [6,7-dimethoxy-1-(3',4'-dimethoxybenzyl)isoquinoline] | 4·0 | 100 | Competitive |
| Ethaverine [6,7-diethoxy-1-(3',4'-diethoxybenzyl)isoquinoline] | 5·6 | 71 | Competitive |
| 1-Benzyl-6,7-dimethoxyisoquinoline | 6·5 | 61 | Competitive |
| 1-(3',4'-Dimethoxybenzyl)isoquinoline | 22 | 18 | Competitive |
| Papaveroline [6,7-dihydroxy-1-(3',4'-dihydroxybenzyl)isoquinoline] | 22 | 18 | Mixed |
| (−)-Glaucine [(−)-1,2,9,10-tetramethoxyaporphine] | 36 | 11 | Mixed |
| (+)-Glaucine [(+)-1,2,9,10-tetramethoxyaporphine] | 39 | 10 | Mixed |
| 6,7-Dimethoxyisoquinoline | 110 | 4 | Mixed |

* The following compounds did not exhibit significant activity at $1 \times 10^{-5}$ M: isoquinoline, (±)-laudanosine [1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-(3',4'-dimethoxybenzyl)isoquinoline], (±)-laudanosoline [1,2,3,4-tetrahydro-6,7-dihydroxy-2-methyl-1-(3',4'-dihydroxybenzyl)isoquionline] 1-benzylisoquinoline, papaverine methiodide, papaveroline methiodide, corydine (1-hydroxy-2,10,11-trimethoxyaporphine), (±)-salsolidine (1,2,3,4-tetrahydro-6,7-dimethoxy-1-methylisoquinoline), (−)-tetrahydropalmitine, (−)-norargenomine, (−)-bisnorargemonine and (±)-*N*-methylpavine.

† $K_i$ values for all compounds were calculated as described in the text employing concentrations of inhibitors in the range of $1 \times 10^{-5}$ to $5 \times 10^{-5}$ M. (±)-Laudanosine was also tested at $1 \times 10^{-4}$ M.

The data in Table 1 show that none of the alkaloids tested is a more effective inhibitor of beef heart PD activity than papaverine. However, from the gradation in effectiveness of the compounds examined, certain tentative conclusions can be drawn concerning the structural requirements for inhibition by papaverine-like agents. The moderate activity of papaveroline, which contains hydroxyl groups in the 6,7 and 3′,4′-positions, and the absence of inhibition by 1-benzylisoquinoline demonstrate the importance of the methoxyl groups for optimal inhibitory activity. The marked difference in effectiveness between 1-benzyl-6,7-dimethoxyisoquinoline and 1-(3′,4′-dimethoxybenzyl)isoquinoline indicates that the 6,7-methoxyl substituents are more critical to activity than the 3′,4′-methoxyl groups. The only compound tested which has inhibitory activity but does not contain a benzyl substituent is 6,7-dimethoxyisoquinoline However, neither 6,7-dimethoxyisoquinoline nor papaveroline appears to act by the purely competitive mechanism seen for the more effective inhibitors.

(+)-Glaucine and (−)-glaucine were the only compounds containing a partially reduced heterocyclic ring which possessed significant inhibitory activity. The identical inhibitory potencies of these stereoisomers indicate an absence of stereoselectivity in the glaucine-promoted inhibition of this PD activity. The inhibitory action of glaucine may be due at least in part to the spatial restrictions imposed by the presence of the biphenyl bond in the aporphine nucleus. (±)-Laudanosine which lacks only the biphenyl linkage of glaucine produced no detectable inhibition.

From these results it can be concluded that the 6,7-methoxyl substituents contribute more to the inhibitory effectiveness of papaverine than the 3′,4′-methoxyl groups. Removal of the 1-benzyl moiety results in a more drastic reduction in activity than removal of either the 6,7 or 3′,4′-methoxyl groups. However, the absence of all four of the methoxyl groups from papaverine renders it inactive. Although the optimal inhibitory activity of papaverine was not surpassed by any agent examined in this study, it is reasonable to expect that other preparations of PD might display different sensitivities to the inhibitors. In this regard, however, Markwardt and Hoffman[6] and Kukovetz and Pöch[7] observed that papaverine was the most effective inhibitor of several substituted isoquinoline derivatives tested with PD preparations from human platelets and beef coronary arteries, respectively. Therefore, papaverine may prove to be a useful tool for uncovering characteristics of the binding and/or active site(s) of the cyclic nucleotide hydrolyzing enzyme(s).

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