Effect of Quazodine on Phosphodiesterase (35357)

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Quazodine (6,7-dimethoxy-4-ethylquinazoline; MJ 1988) has been reported to possess significant bronchodilator, vasodilating, and cardiac stimulating properties (1, 2). It seems to produce its effects directly and not by way of the autonomic nervous system (1–3). The reported (2) pharmacologic profile of quazodine resembles that of theophylline and the data suggest that the former is the more potent compound. Also quazodine is well absorbed from the gastrointestinal tract (2). Since inhibition of phosphodiesterase (PDE) seems to be the underlying mechanism for most of the pharmacologic effects of theophylline (4), it was of interest to study the effect of quazodine on the activity of that enzyme isolated from a number of tissues. The chemical structures of quazodine and theophylline are shown in Fig. 1.

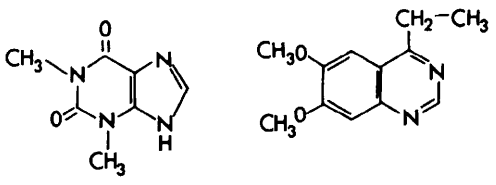

FIG. 1. Structures of theophylline and quazodine.

Materials and Methods. Male albino rabbits, weighing 1.9–3.5 kg each, were fasted for 15 hr prior to sacrifice. The rabbits were stunned by rapid cervical dislocation and the tissues were quickly excised and placed in ice-cold 0.25 *M* sucrose solution. The tissues were then homogenized in the sucrose solution and diluted to yield a 20% homogenate based on the tissue wet weight. The homogenate was centrifuged at 4000g; and PDE in the supernatant fluid was partially purified by ammonium sulfate fractionation as described by Nair (5).

Beef brains obtained from a local slaughterhouse were used as a source of brain PDE, which was partially purified by ammonium sulfate fractionation as described by Cheung (6).

PDE activity was assayed according to the method described by Butcher and Sutherland (7) with slight modifications. At least four different substrate concentrations were used to determine the Michaelis constant and the maximal velocity. The incubation mixture contained 0.09–0.72 μmole of cyclic 3′, 5′-AMP and 1.8 μmoles of $MgSO_4$ in addition to either quazodine or theophylline which was present in a final concentration of 10^{-5} to 10^{-3} *M*. Incubations were carried out for 30 min at 30°. Inorganic phosphate was released from the resulting AMP by the addition of *Crotalus atrox* venom and the phosphate was assayed colorimetrically (8). One unit of PDE activity was defined as that amount of enzyme activity which hydrolyzed 1 μmole of cyclic 3′,5′-AMP in 30 min at 30° (7).

The Michaelis constant, K_m, was calculated by a computer program designed according to the method described by Mounter and Turner (9). The inhibitor constant, K_i, was calculated according to the following formula:

$$K_i = \frac{[i]}{(V/V_i) - 1}$$

where [i] = molar inhibitor concentration; V = maximal enzyme velocity; and V_i = apparent maximal velocity in presence of the inhibitor.

Results. The results obtained with partially purified PDE isolated from a number of rabbit tissues and from bovine brain are included in Table I. In all the tissues examined, both quazodine and theophylline be-

TABLE I. Effect of Quazodine and Theophylline on Partially Purified Phosphodiesterase Isolated from Selected Rabbit Tissues and Bovine Brain.[a]

Tissue	Sp act [U[b]/g tissue (wet wt)]	K_m[c] ($M \times 10^{-3}$)	K_i[d]		
			Quazodine	Theophylline	T/Q
Rabbit lung	16.70	0.226	3.61	8.96	2.5
heart	6.25	0.223	13.31	22.80	1.7
liver	22.40	0.289	1.81	3.22	1.8
spleen	30.30	0.303	15.4	18.3	1.2
kidney	58.60	0.364	8.0	15.1	1.9
pyloric mucosa	6.95	0.199	12.9	21.9	1.7
fundic mucosa	90.90	0.414	7.73	10.1	1.3
Bovine brain	123.30	0.114	1.16	7.52	6.5

[a] All values are the averages of 3–6 expts.

[b] 1 U = the amount of enzyme activity that caused the destruction of 1 μmole of cyclic 3',5'-AMP in 30 min at 30°.

[c] K_m = Michaelis constant.

[d] K_i = inhibitor constant ($\times 10^{-4}$).

haved as noncompetitive inhibitors of the enzyme. An example is shown in Fig. 2 for the enzyme isolated from rabbit pyloric mucosa. Based on the ratio of the K_i for each agent the PDE inhibiting potency of quazodine varied from 1.1 to 6.5 times that of theophylline.

Discussion. The present data indicate that both compounds acted as noncompetitive inhibitors of PDE. This is consistent with the report by Nair (5) describing the noncompetitive inhibition by caffeine of PDE from the dog heart. However neither our finding nor that of Nair is consistent with that of Butcher and Sutherland (7), who reported that methyl xanthines competitively inhibited PDE isolated from beef heart. It is not clear whether the divergent results are due (a) simply to the fact that there is no common PDE source in these studies, or (b) to some more subtle difference in the properties of the various enzyme preparations. Possibly an unobvious, minor difference in the isolation and preparative methods from one laboratory to another might account for such a cryptic difference in the properties of the enzyme.

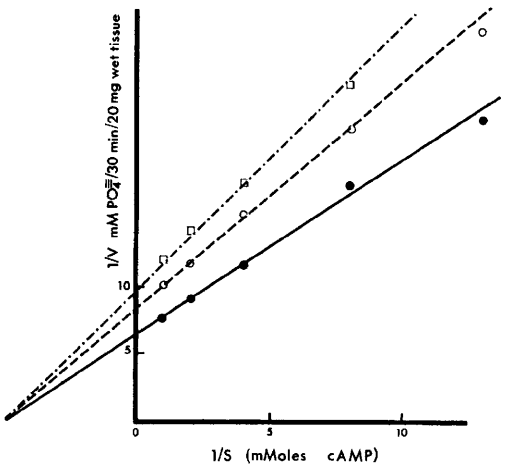

FIG. 2. Lineweaver-Burk plot of the effect of quazodine, 10^{-4} M (□); and theophylline, 10^{-4} M (○--); on the activity of partially purified cAMP phosphodiesterase from rabbit pyloric mucosa (●—).

Although quazodine is a substituted quinazoline it has a striking structural similarity to the xanthines in general and to theophylline in particular, especially to the relative position of the two nitrogens (Fig. 1). Studies in progress in our laboratories indicate that indeed the —N—C=N— structure is essential for both inhibition and stimulation (imidazole) of PDE, whereas the remainder of the molecule seems to play only a relatively minor role.

The inhibition of PDE by both quazodine and theophylline and the similarity of their pharmacologic profiles suggest that such effect could be the underlying mechanism of action of each compound. However, as with theophylline (10), this mechanism may not

totally account for all the effects of quazodine.

Summary. Quazodine (6,7-dimethoxy-4-ethylquinazoline, MJ 1988) was up to 6 times as potent as theophylline in inhibiting PDE partially purified from a number of rabbit tissues and beef brain. As with theophylline, inhibition of PDE could be the underlying mechanism for the bronchodilator and cardiovascular effects of quazodine.

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