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# Inhibition by etazolate (SQ 20009) and cartazolate (SQ 65396) of adenosine-stimulated [$^3$H]cAMP formation in [2-$^3$H]adenine-prelabeled vesicles prepared from guinea pig cerebral cortex

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Etazolate (SQ 20009) is a potent phosphodiesterase inhibitor once believed to increase cyclic AMP in brain and thus to account for its anxiolytic action in animal conflict procedures [1]. *In vitro* it caused approximately 50% inhibition of cyclic AMP-phosphodiesterase activity of subcellular preparations of rat brain [1–3], rabbit brain [3], rat liver [4] and human lung [5] at concentrations from 2 to 7 μM. At higher concentrations it also inhibited the enzyme activity of subcellular preparations of liver fluke [6], Syrian hamster islet cell tumor [7], rat lung [8] and canine tracheal smooth muscle [9]. However, etazolate did not increase cyclic AMP in guinea pig brain cortical slices *in vitro* except in the presence of an agonist such as histamine [10], and *in vivo* it did not significantly increase cyclic AMP in rat brain although it increased cyclic AMP in plasma and urine [11]. Moulin and Schultz [12] concluded that it was difficult to correlate phosphodiesterase-inhibitor potency with pharmacological action, and an alternative mechanism of action for etazolate and a related drug cartazolate (SQ 65396) was proposed on the basis of their enhancement of diazepam binding to brain membrane preparations [13, 14].

In the present study, etazolate and cartazolate were tested as adenosine antagonists since it is thought that some phosphodiesterase inhibitors, such as theophylline, produce some of their pharmacological effects principally by antagonizing adenosine [15]. Previous studies had shown etazolate to be either inactive [16] or only slightly active [10] in inhibiting adenosine-stimulated adenylate cyclase in guinea pig cortical slices, but a high concentration of adenosine had been used. Preliminary studies in our laboratory on adenylate cyclase of vesicle preparations from guinea pig cerebral cortex stimulated by low concentrations of adenosine, in keeping with the adenosine concentration in brain [17], showed that, as adenosine antagonists, etazolate and cartazolate were at least as potent as theophylline.

[2-$^3$H]Adenine-prelabeled vesicles were prepared from guinea pig cerebral cortex, and adenylate cyclase activity was measured as described previously [18, 19]. Cartazolate was donated by Mr. S. Lucania of the Squibb Institute (Princeton, NJ) and 8-*p*-sulfophenyltheophylline was donated by Dr. J. Daly of the National Institutes of Health (Bethesda, MD). Etazolate was prepared by Mr. R. Dziemian, and 8-*p*-bromophenyltheophylline was prepared by Dr. F. Clarke and Mr. W. Heiber of these laboratories. 8-Phenyltheophylline was obtained from the CalBiochem-Behring Corp. (La Jolla, CA), adenosine from Schwarz/Mann (Orangeburg, NY), theophylline from Nutritional Biochemicals (Cleveland, OH), and caffeine from the Sigma Chemical Co. (St. Louis, MO).

Figure 1 shows that etazolate, cartazolate, theophylline and caffeine displaced the adenosine concentration–activity curves in a manner consistent with a competitive action at the adenosine receptor; approximate $K_i$ values, based on concentrations of adenosine necessary for half-maximum activation in the absence and presence of antagonist, were 7.2, 5.6, 19 and 45 μM respectively. Lower $K_i$ values, similar to those reported on adenylate cyclase activity of VA13 human fibroblast cells [20, 21], were calculated from $IC_{50}$ values using a low, fixed concentration of adenosine (Table 1). The effects of other standard adenosine antagonists are also shown in Table 1.

The potency of etazolate as an adenosine antagonist ($K_i$ = 7.2 μM, Fig. 1; 3.9 μM, Table 1) is similar to its potency ($K_i$ = 4 μM) as an inhibitor of the low-$K_m$ cyclic AMP-phosphodiesterase of rat liver [4] and greater than its potency as an inhibitor of the high-$K_m$ phosphodiesterase ($K_i$ = 70 μM [4]. The concentration required for enhancement of diazepam binding [14] is in the same range as the $IC_{50}$ value for inhibition of adenosine activation of adenylate cyclase. The ability of the drug to antagonize adenosine may thus be related to some of its pharmacological actions. For example, its high potency in relaxing tracheal smooth muscle could not be explained by its relatively low potency in inhibiting phosphodiesterase activity of this tissue [9] and thus might be attributed to its ability to antagonize endogenous adenosine. Likewise, adenosine antagonism might be an underlying molecular mechanism in certain pharmacological effects of cartazolate.

Following the completion of these studies, tracazolate (ICI 136753), a methyl-substituted analog of cartazolate, was reported to inhibit binding of [$^3$H]cyclohexyladenosine to homogenate preparations of rat testes and brain with a potency considerably greater than that of theophylline [24]. We also found tracazolate to be effective in our system but

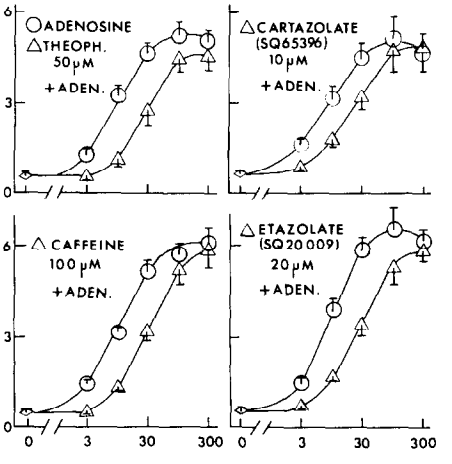


Fig. 1. Inhibition of adenosine-activated adenylate cyclase in [2-$^3$H]adenine-prelabeled vesicles by theophylline, caffeine, cartazolate (SQ 65396) and etazolate (SQ 20009). [2-$^3$H]Adenine-prelabeled vesicles corresponding to one-fortieth of a guinea pig cerebral cortex were incubated for 15 min at 37° with adenosine and antagonists at the concentrations shown above by the general procedure described previously [18, 19]. Each point is the mean of three separate experiments, each performed in triplicate; the S.E.M. is also shown. $K_i$ values calculated from concentrations of adenosine required for half-maximum activation in the absence ($K_a$) and presence ($K_a'$) of antagonist at a single concentration ($I$) by the formula $K_i = [I/(K_a'/K_a) - 1]$ (see Ref. 22) were 45 μM (caffeine), 19 μM (theophylline), 7.2 μM (etazolate) and 5.6 μM (cartazolate); mean $K_a$ values for adenosine in these experiments were 8.9, 9.0, 7.8 and 9.7 μM respectively.

Table 1. $IC_{50}$ Values of standard adenosine antagonists, etazolate (SQ 20009) and cartazolate (SQ 65396), on adenosine-activated adenylate cyclase of [2-$^3$H]adenine-prelabeled vesicles*

| Antagonist | Brain vesicles $IC_{50}$ ($\mu$M) | Brain vesicles $K_i$ ($\mu$M) | VA13 human fibroblast cells $K_i$ ($\mu$M) |
|---|---|---|---|
| Caffeine | 25 | 16 | 13 |
| Theophylline | 10 | 6.4 | 4.8 |
| 8-*p*-Sulfophenyltheophylline | 2.8 | 1.8 | 1.2 |
| 8-Phenyltheophylline | 0.47 | 0.30 | 0.18 |
| 8-*p*-Bromophenyltheophylline | 0.15 | 0.09 | 0.05 |
| Etazolate (SQ 20009) | 6.2 | 3.9 | |
| Cartazolate (SQ 65396) | 3.1 | 2.0 | |

* $IC_{50}$ Values were obtained by testing antagonists at three or more different concentrations against a fixed concentration (5 $\mu$M) of adenosine. $K_i$ values were calculated by the formula (see Ref. 22) $K_i = IC_{50}/1 + S/K_a$ in which $S$ is the concentration of adenosine and $K_a$ is the concentration of adenosine required for half-maximum activation (mean value was 8.8 $\mu$M). $K_i$ values for VA13 human fibroblast cell adenylate cyclase were taken from literature reports [20, 21]; they were obtained essentially by measuring inhibition against a fixed concentration (10 $\mu$M) of adenosine [23].

less potent than cartazolate, with an $IC_{50}$ value of 20 $\mu$M. Tracazolate was donated by Dr. D. H. McCurdy of ICI Americas, Inc. (Wilmington, DE).

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*Research Department*
*Pharmaceuticals Division*
*CIBA-GEIGY Corp.*
*Ardsley, NY 10502, U.S.A.*

STACY PSYCHOYOS*
CATHERINE J. FORD
MILDRED A. PHILLIPPS

REFERENCES

1. Z. P. Horovitz, B. Beer, D. E. Clody, J. R. Vogel and M. Chasin, *Psychosomatics* **13**, 85 (1972).
2. M. Chasin, D. E. Clody, J. R. Vogel and Z. P. Horovitz, *Science* **176**, 428 (1972).
3. M. Chasin, D. N. Harris, M. B. Phillips and S. M. Hess. *Biochem. Pharmac.* **21**, 2443 (1972).
4. C. Ingebretsen, J. F. Clark, D. O. Allen and J. Ashmore, *Biochem. Pharmac.* **23**, 2139 (1974).
5. W. F. Glass and J. B. Moore, Jr., *Biochem. Pharmac.* **28**, 1107 (1979).
6. T. E. Mansour and J. M. Mansour, *Biochem. Pharmac.* **26**, 2325 (1977).
7. U. K. Schubart, L. Udem, S. G. Baum and O. M. Rosen, *Molec. cell. Endocr.* **1**, 227 (1974).
8. R. Fertel and B. Weiss, *Molec. Pharmac.* **12**, 678 (1976).
9. J. B. Polson, J. J. Krzanowski, D. F. Fitzpatrick and A. Szentivanyi, *Biochem. Pharmac.* **27**, 254 (1978).
10. J. Schultz, *Archs. Biochem. Biophys.* **163**, 15 (1974).
11. I. Weinryb, B. Beer, M. Chasin, E. B. Proctor and S. M. Hess, in *Excerpta Med. Int. Congr. Ser. No. 359, Neuropsychopharmacology* (Eds. J. R. Boissier, H. Hippins and P. Pinchot), pp. 857–65. Elsevier, Amsterdam (1975).
12. A. Moulin and J. Schultz, *Experientia* **31**, 883 (1975).
13. B. Beer, C. A. Klepner, A. S. Lippa and R. F. Squires, *Pharmac. Biochem. Behav.* **9**, 849 (1978).
14. M. Williams and E. A. Risley, *Life Sci.* **24**, 833 (1979).
15. B. F. Fredholm and P. Hedqvist, *Biochem. Pharmac.* **29**, 1635 (1980).
16. H. D. Mah and J. W. Daly, *Pharmac. Res. Commun.* **8**, 65 (1976).
17. M. Newman and H. McIlwain, *Biochem. J.* **164**, 131 (1977).
18. S. Psychoyos, *Life Sci.* **23**, 2155 (1978).
19. S. Psychoyos, *Biochem. Pharmac.* **30**, 2182 (1981).
20. R. F. Bruns, *Soc. Neurosci. Abstr.* **4**, 442 (1978).
21. R. F. Bruns, J. W. Daly and S. H. Snyder, *Proc. natn. Acad. Sci. U.S.A.* **77**, 5547 (1980).
22. P. D. Kanof and P. Greengard, *J. Pharmac. exp. Ther.* **209**, 87 (1979).
23. R. F. Bruns, *Biochem. Pharmac.* **30**, 325 (1981).
24. K. M. M. Murphy and S. H. Snyder, *Life Sci.* **28**, 917 (1981).

* Author to whom correspondence should be addressed.