Activity of Parthenolide at $5HT_{2A}$ Receptors

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Parthenolide displaces [^{3}H]ketanserin from $5HT_{2A}$ receptors from rat and rabbit brain and cloned $5HT_{2A}$ receptors. K_i's are in the 100−250 μM range. These results suggest that parthenolide may be a low-affinity antagonist at 5HT receptors; it is unlikely that the entire mechanism of action can be explained by its modest $5HT_{2A}$ receptor affinity.

Parthenolide, a sesquiterpene lactone, is the putative active principle of feverfew (*Tanacetum parthenium*; (L.) Schultz-Bip., Asteraceae).[1−3] Feverfew has been widely used as a herbal remedy, especially for the prophylactic treatment of migraine headache. Although migraine headache is a complex neurovascular disorder, recent evidence suggests that serotonin (5-hydroxytryptamine; 5HT) receptor-based mechanisms are central to its pathophysiology.[4] 5HT receptors have become highly characterized biochemically and pharmacologically in the last two decades,[5,6] and methodology is available for characterization of drug-receptor binding.

Multiple subtypes of 5HT receptors are currently recognized.[5,6] Of these, antimigraine drugs are thought to interact predominantly with receptors of the $5HT_1$ and $5HT_2$ classes.[4] $5HT_2$ receptors are found as three distinct subtypes, $5HT_{2A,2B,2C}$.[5,6] Of particular interest to this paper, $5HT_{2A}$ receptors are found in numerous locations peripherally, including the vasculature and in central locations such as cerebral cortex, limbic system, and basal ganglia.

Controversy exists regarding the biochemical mechanism of action for parthenolide as an antimigraine drug.[1−3] On the one hand, evidence from platelets supports the idea that feverfew and parthenolide, which is one of the major components, exert antimigraine action via a 5HT receptor-based mechanism.[1] Additional studies with platelets demonstrated the activity of parthenolide against a variety of platelet stimulants but did not directly implicate serotonergic mechanisms;[2] however, it was suggested that the protein kinase C pathway might represent the basis of action.

Evidence from a different biological system, aortic rings, suggested that parthenolide-rich extracts of fresh feverfew are active inhibitors of vascular smooth muscle contractions.[3] This effect is nonspecific, however, and the exact mechanisms of inhibition are unclear. In the same study, chloroform extracts of dried feverfew produced vascular contraction, rather than inhibition; this activity was not antagonized by ketanserin, a $5HT_{2A}$ antagonist. Recently, the structure−activity relationships of parthenolide and related sesquiterpene lactones has been reviewed,[7] with evidence set forward that these compounds primarily inhibit the release of serotonin.

In light of these intriguing experimental findings, we thought it of interest to test parthenolide's activity against $5HT_{2A}$ receptors, which are known to be relevant to some processes in platelets and vascular smooth muscle.[6,8] The studies reported here test the activity of parthenolide at $5HT_{2A}$ receptors in a direct *in vitro* format. We utilize $5HT_{2A}$ receptors from two well-characterized species, rabbit[8] and rat.[9] Results are reported on the extent to which parthenolide displaces [^{3}H]ketanserin from $5HT_{2A}$ receptors in these two species. A preliminary report of these results has appeared.[10]

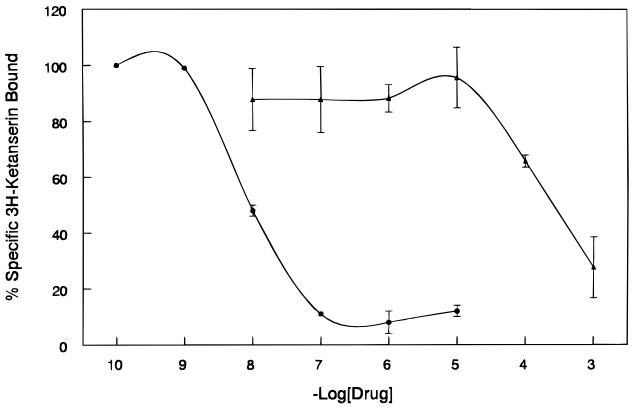

Figure 1. Displacement of specific [^{3}H]ketanserin binding from rabbit cerebral cortex membranes. Results shown are mean ± standard error of the mean for three determinations. [^{3}H]Ketanserin concentration was 0.25 nM. Other experimental conditions and calculations were described in the Experimental Section. Displacing drugs: methysergide (●); parthenolide (▲).

Parthenolide displaces the $5HT_{2A}$ antagonist, [^{3}H]-ketanserin, in a concentration-dependent manner in membranes prepared from rabbit cerebral cortex (Figure 1). For comparative purposes, the activity of parthenolide is shown in relation to the high affinity ergot antagonist, methysergide. In a parallel series of experiments, the activity of parthenolide was compared to another high affinity antagonist, mesulergine, yielding similar results (not shown in Figure 1). Using the Cheng/Prusoff transformation[11] to convert IC_{50}'s to K_i's, the following affinity values were obtained: methysergide, 5.7 ± 1.5 nM; mesulergine, 8.3 ± 0.7 nM; parthenolide, 230 ± 90 μM. The highest concentration of parthenolide used (1 mM) included a final concentration of 2% ethanol in reaction tubes. Control experiments showed no effect of ethanol at or below this concentration; however, at concentrations above 2% ethanol, concentration-dependent inhibition was observed in the assay. Thus, ethanol was never included at levels higher than 2% in experiments utilizing parthenolide. To address concerns that parthenolide might not be reaching receptors because of solubility considerations,

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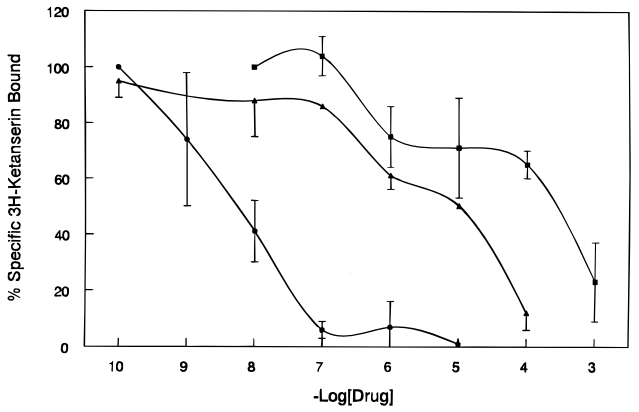

Figure 2. Displacement of specific [^{3}H]ketanserin binding from cloned rat $5HT_{2A}$ receptor. Results shown are mean ± standard error of the mean for three determinations, except for spiperone, where $n = 4$. [^{3}H]Ketanserin concentration was 0.25 nM. Other experimental conditions and calculations were described in the Experimental Section. Displacing drugs: spiperone (●); (−)-propranolol (▲); parthenolide (■).

a series of experiments were run in which the detergent Tween was added at 0.1% final concentration in all assay tubes. This addition did not change the concentration-dependent displacement curves of parthenolide.

Figure 2 compares parthenolide's capacity to displace [^{3}H]ketanserin with the high affinity antagonist spiperone in membranes derived from clonal rat $5HT_{2A}$ receptor. Additionally, the β blocker, (−)-propranolol, which shows moderate to low affinity for $5HT_{2A}$ receptors,[5,6] was included for comparative purposes. Affinity values for these agents were as follows: spiperone, 1.0 ± 0.1 nM; (−)-propranolol, 8.1 ± 0.6 μM; parthenolide, 100 ± 11 μM. For comparative purposes, we conducted a limited number of experiments in which parthenolide was used to displace [^{3}H]ketanserin from membranes prepared from rat cerebral hemispheres. The K_i for parthenolide was 250 μM.

As demonstrated in Figures 1 and 2, parthenolide gives very modest displacement of [^{3}H]ketanserin from membranes derived from rabbit cerebral cortex as well as membranes derived from NIH 3T3 cells expressing the cloned rat $5HT_{2A}$ receptor. Activity at the $5HT_{2A}$ receptor may contribute to the pharmacological mechanism of action for parthenolide. Nevertheless, the K_i's reported here for parthenolide at $5HT_{2A}$ receptors from rabbit and rat and the cloned rat $5HT_{2A}$ receptor, which are in the 100−250 μM range, rank parthenolide with $5HT_{2A}$ antagonists which are generally considered to be low affinity.[5,6] While low-affinity agents may well interact with a receptor physiologically, it is less likely that such low-level receptor activity can explain their biological activity completely.

The results reported here support, in part, the hypothesis that parthenolide is a 5HT antagonist.[1,2] The weak activity of parthenolide at $5HT_{2A}$ receptors, however, suggests that other targets may be equally or even more important than $5HT_{2A}$ receptors. We have previously reported that $5HT_{1A}$ receptors are not targets for parthenolide.[12] Other 5HT receptors need to be screened as well as other aspects of 5HT receptor systems, downstream from the ligand binding site in the signal transduction system. A report utilizing rat stomach fundus contractile responses as an experimental model suggests that $5HT_{2B}$ receptors are not inhibited by parthenolide while supporting the hypothesis that the drug does inhibit serotonin release mechanisms.[13] Mechanisms outside the 5HT realm also need to be considered.

It is possible that other compounds, yet to be identified from feverfew extracts, contribute to the biological activity of parthenolide and related sesquiterpenes. It is more than feasible that a number of compounds are contributing to feverfew's medicinal activity via multiple mechanisms. Of these compounds, parthenolide may play a role, partially mediated by a $5HT_{2A}$ receptor-based mechanism.

Experimental Section

Materials. Standard buffers and laboratory chemicals were of reagent grade and were from either J. T. Baker (Phillipsburg, NJ) or Sigma Chemical Co. (St. Louis, MO). Displacing drugs were from Research Biochemicals International (Natick, MA). Parthenolide was from Aldrich Chemical Co. (Milwaukee, WI); parthenolide stock was monitored spectrometrically for integrity. Tissue culture reagents were from GIBCO/BRL Life Technologies (Gaithersburg, MD). [^{3}H]Ketanserin was from NEN (Boston, MA).

Preparation of Brain Tissue and Cell Culture. New Zealand White rabbits were anesthesized with pentobarbital, and the brains were rapidly removed at 4 °C, dissected, and immediately frozen in liquid nitrogen. Tissue was maintained at −70 °C until used. For receptor binding studies, tissue was thawed in 40 volumes of Tris buffer (50 mM, pH 7.4) at 4 °C and then homogenized with a Polytron (setting 5, 30 s). Homogenate was centrifuged at 4 °C for 450 000 g/min. The resulting pellet was resuspended in Tris buffer, rehomogenized, and recentrifuged. Rat brain tissue was handled in a similar manner, except that rats were not anesthetized prior to sacrifice.

NIH 3T3 cells expressing the rat $5HT_{2A}$ receptor[9] (gift of Dr. David Julius, UCSF) were cultured in DMEM containing 200 μg/mL of geneticin and fortified with 10% calf serum. Cultures were incubated at 37 °C, in a humidified atmosphere of 5% CO_2. Cells were harvested in 0.25% trypsin, and the reaction was stopped in appropriate medium followed by centrifugation for 10 000 g/min at 4 °C. The pellet was resuspended in Earle's balanced salt solution and recentrifuged. The resulting pellet was homogenized in glass with Tris buffer, and handled as above for brain tissues.

Binding Assays. Binding of [^{3}H]ketanserin (85 Ci/mmol) to rabbit or rat cerebral cortical membranes was conducted at 30 °C for 20 min using modifications of techniques previously described.[8,14−16] The procedure involved resuspension of the final membrane pellet in 80 volumes of Tris buffer containing 4 mM $CaCl_2$ and 10 μM pargyline. Each sample contained 700 μL of homogenate, 100 μL of buffer or 100 μL of nonspecific blocker (10 μM mesulergine) in buffer, 100 μL of displacing drug in buffer, and 100 μL of radiolabeled agent. The final volume was 1 mL. Assays of receptors from cultured cells were identical except that the incubation time was extended to 30 min.

Due to solubility considerations, parthenolide was first dissolved in absolute ethanol followed by dilution in binding buffer. Following incubation, reactions were stopped by addition of 4 mL of ice-cold Tris buffer and

rapid vacuum filtration through glass fiber filters (Whatman GF/B or S & S #32); filters were then rinsed with two additional 5 mL aliquots of ice-cold buffer. Filters were counted in 5 mL of Ecoscint scintillation fluid (National Diagnostics) in a Beckman liquid scintillation system (LS 6500). All determinations were in duplicate or triplicate and were conducted on three separate occasions unless otherwise indicated. Measurement of homogenate protein content utilized the colorimetric procedure of Bradford[17] in the microformat with albumin as standard. Binding parameters were analyzed by computerized procedures (PC NON-LIN (Statistical Consultants Inc., KY) and KCAT (Biometallics Inc., NJ)) as well as fitted by hand. K_i's were determined from IC_{50}'s as described by Cheng and Prusoff.[11]

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