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CARBACHOL STIMULATION OF PHOSPHATIDIC ACID SYNTHESIS: COMPETITIVE INHIBITION BY PIRENZEPINE IN SYNAPTOSOMES FROM RAT CEREBRAL CORTEX

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Carbachol stimulated phosphatidic acid synthesis in cholinergically enriched synaptosomes from rat cerebral cortex. Increasing concentrations of pirenzepine (10-1000 nM) produced parallel concentration-response curves to carbachol which were shifted to the right. A pA_2 value for pirenzepine of 8.4 $\pm$ 0.3 was obtained from Schild analysis. We hypothesize that high affinity pirenzepine binding to M_1 receptors is coupled to phosphatidic acid synthesis in the rat cerebral cortex.

Recent physiological, pharmacological and biochemical data support the hypothesis that muscarinic cholinergic receptor subtypes (M_1 and M_2), exist in the central nervous system and periphery, (1-8) and that the recognition sites for these receptors are differentially regulated most likely due to effector coupling (9-11). A drug that has played a significant role in the demonstration of muscarinic cholinergic receptor heterogeneity is pirenzepine, a non-classical muscarinic antagonist which does not pass the blood brain barrier and therefore, is useful as an anti-ulcer drug (12). A radiolabeled form of pirenzepine has been used to demonstrate and characterize muscarinic receptor heterogeneity in the rat cerebral cortex, ileum and heart (3-5,13,14). Thus, the high affinity dissociation constant (Kd = 2-10nM) in these tissues is thought to be associated with the M_1 receptor and the low affinity dissociation constant (Kd = 1uM) associated with the M_2 receptor. Since, in the cerebral cortex these two subtypes of muscarinic receptors have been primarily characterized on the basis of radioligand binding studies, we

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thought it would be desirable to provide further evidence of muscarinic receptor subtypes on the basis of an associated biochemical response.

It has been known for some time that in neural preparations, acetylcholine or carbamylcholine can stimulate the incorporation of (^{32}P) into phosphatidic acid (PhA) and phosphatidylinositol (PhI) (15,16). By comparing the muscarinic phospholipid responses in cholinergically enriched synaptosomes from rat cerebral cortex in the absence and presence of varying concentrations of pirenzepine and performing a Schild analysis (17) we have demonstrated both the competitive nature of the pirenzepine antagonism as well as the muscarinic subtype associated with the receptor-mediated phosphatidic acid synthesis. We have presented initial results previously (18).

METHODS

Adult male Sprague-Dawley rats were killed by decapitation. The cerebral cortex was rapidly excised and homogenized in 10 vols ice-cold 0.32M sucrose. "Cholinergically enriched" synaptosomes (1.2-1.0 M sucrose interface) were isolated on a 4-step sucrose density gradient (19), washed, and resuspended in a phosphate-free Krebs Ringer HEPES medium, pH 7.4.

[^{32}P] incorporation into phospholipids: Aliquots (100ul) of synaptosomes (200-300ug protein) were added to tubes containing 100ul [^{32}P] (5uCi/tube) in the same buffer plus indicated drugs. Incubations were run in triplicate at 37° for 60 minutes and stopped with the addition of 3.8ml $CHCl_3$:CH_3OH (2:1). The resulting total lipid extract was washed repeatedly (20). Phosphatidic acid (PhA) was isolated by one-dimensional thin-layer chromatography, scraped and counted for radioactivity as described earlier (21). Results are expressed as percent of control (basal) values and are the mean S.E.M. from 3-5 separate experiments.

RESULTS AND DISCUSSION

The incorporation of [^{32}P] Pi into phosphatidic acid of synaptosomes harvested from the 1.0 - 1.2 M sucrose interface was stimulated in the presence of carbamylcholine. This stimulation varied with the concentration of carbamylcholine used and was essentially maximal at 1 mM with an ED_{50} value for carbamylcholine of approximately 100 uM (Fig. 1). The results are in close agreement with a previous report in which synaptosomes from guinea pig cerebral cortex were used (22). Since increasing concentrations of pirenzepine produced parallel shifts to the right in the concentration -response curve and since inhibition by pirenzepine at each concentration tested could be fully reversed by higher concentrations of carbamylcholine, we

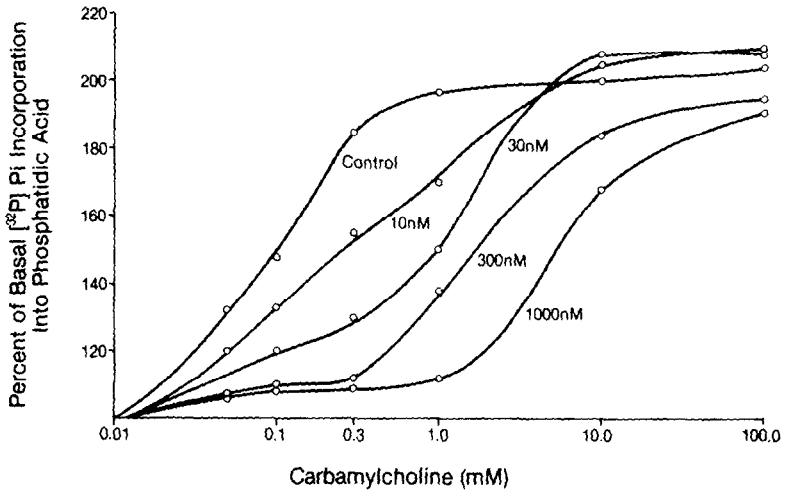

Figure 1. Effect of increasing concentrations of pirenzepine at 10 nM, 30 nM, 300 nM, 1000 nM on carbachol stimulation of phosphatidic acid synthesis in rat cerebral cortical synaptosomes.

conclude that the inhibition is competitive in nature. From the data in Fig. 1, a Schild's plot was constructed (Fig. 2). The straight line in Fig. 2 was generated by least squares analysis and yielded a pA_2 value for pirenzepine of 8.4 $\pm$ 0.3. This value is very similar to that obtained with [^{3}H]pirenzepine from radioligand binding studies in which membrane fragments from crude homogenates of rat cerebral cortex were used (5). Therefore, the results of the present investigation strongly suggest that the high affinity pirenzepine binding sites (M_1 muscarinic receptor subtype) in the cerebral cortex are coupled to phosphatidic acid synthesis. Similar conclusions have been reached by investigators using other preparations (23,24). The biochemical or physiological relevance of M_1-receptor-mediated phospholipid turnover in synaptosomes is yet to be established.

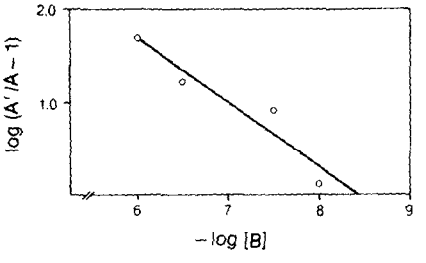

Figure 2. Schild plot of the effect of pirenzepine on carbachol stimulation of phosphatidic acid synthesis. The pA_2 value of pirenzepine was 8.4. $\pm$ 0.3.

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