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Quinacrine and 2-(4-Phenylpiperidino)cyclohexanol (AH5183) Inhibit Acetylcholine Release and Synthesis in Rat Brain Slices

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Summary

The effects of 2-(4-phenylpiperidino)cyclohexanol (AH5183) and quinacrine, two potent inhibitors of acetylcholine transport into vesicles isolated from *Torpedo* electric organ, were examined on acetylcholine metabolism in rat cortical slices. K^+-stimulated acetylcholine release was reduced in a concentration-dependent manner by AH5183 and quinacrine, with IC_{50} values of 1 μM and 50 μM, respectively. Both drugs also reduced the synthesis of acetylcholine in slices and inhibited synaptosomal high affinity choline transport. The inhibitory effect of AH5183 appears to be directed primarily on the release of acetylcholine while the major effect of quinacrine is on the synthesis of acetylcholine. Examination of the subcellular distribution of acetylcholine in brain slices incubated in high K^+ showed that AH5183 increased S_3 (cytoplasmic) acetylcholine levels but did not alter P_3 (vesicular) acetylcholine levels. P_3 acetylcholine levels were reduced by AH5183 in a low K^+ media while the S_3 acetylcholine levels were the same as controls. These results are consistent with the concept that there is a small, active, highly labile fraction of vesicles that are the source of the released acetylcholine and that the loading of these vesicles is blocked by AH5183.

Recently, Anderson *et al.* (1) studied a number of potential inhibitors of acetylcholine transport into purified vesicles isolated from the electric organ of *Torpedo*. The two most potent inhibitors that were identified are AH5183 (IC_{50} = 40 nM) and quinacrine (IC_{50} = 400 nM). AH5183 had previously been shown to cause neuromuscular blockage (2), and Marshall (3, 4) concluded that AH5183 was acting by inhibiting vesicular loading of acetylcholine because the blockade developed slowly, was enhanced by increased release of acetylcholine, and was not antagonized by choline, neostigmine, or tetraethylammonium. The site of action of AH5183 was demonstrated more directly by Toll and Howard (5) and Melega and Howard (6) who reported that it inhibited the loading of newly synthesized acetylcholine into granules in PC12 cells without altering the vesicular content of previously loaded acetylcholine.

Quinacrine, the second most potent inhibitor of acetylcholine transport into purified vesicles that was reported by Anderson *et al.* (1), has been reported to inhibit a number of processes, most notably phospholipase A_2 activity (7), calcium flux (8), and neurotransmitter release (9–11), but none of these processes was as sensitive to quinacrine as was vesicular acetylcholine transport.

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These two most potent inhibitors of acetylcholine transport into purified *Torpedo* vesicles were chosen for the present study with three goals: (a) to determine if they have effects on acetylcholine release from brain tissue, (b) to study the specificity of their effects, and (c) to determine whether AH5183 alters the subcellular distribution of acetylcholine in brain tissue.

Experimental Procedures

Materials

Chemicals. Quinacrine was obtained from Sigma Chemical Co., St. Louis, MO, and AH5183 was a gift from Glaxo Export Ltd. Deuterated variants of acetylcholine and choline were generously provided by Dr. Donald Jenden, UCLA.

Methods

Brain slices. Adult, male Sprague-Dawley rats were decapitated, the brains placed in ice-cold sucrose, the cerebral cortices rapidly dissected and sliced (0.3 mm) in two perpendicular directions using a McIlwain tissue slicer. The slices were preincubated in Krebs-phosphate buffer (NaCl, 122 mM; $MgCl_2$, 1.2 mM; KCl, 4.9 mM; $CaCl_2$, 1.3 mM; NaH_2PO_4, 5.8 mM; dextrose, 11 mM; pH 7.4) for 60 min at 37° to restore energy balance and then washed several times with buffer. Slices were incubated in duplicate or triplicate in buffer containing 4.9 mM or 50 mM KCl (NaCl adjusted), 20 μM paraoxon (to inhibit acetylcholinesterase), and 10 μM [$^2H_4$]choline ($((CH_3)_3NCD_2CD_2OH)$

ABBREVIATIONS: AH5183, 2-(4-phenylpiperidino)cyclohexanol; GCMS, gas chromatography/mass spectrometry.

for 15 min at 37°. Incubations were terminated by addition of cold saline followed by rapid centrifugation. The resulting supernatants and pellets were extracted as described below and the concentrations of labeled and unlabeled acetylcholine were determined by GCMS.

Acetylcholine determinations. Acetylcholine was extracted by homogenizing the tissue in 3 vol of cold 15% 1 N formic acid, 85% acetone containing an internal standard in a precisely known quantity. The incubation medium was acidified and internal standard added. [$^2H_9$]Acetylcholine (containing 9 deuterium atoms in the *N*-methyl moiety) was used as internal standard. Previous work has shown that this isotopic variant is extracted and partitioned in the gas chromatograph identically with the unlabeled variant making it an ideal compound to be used as an internal standard (12). The homogenate was centrifuged (10,000 × *g*, 20 min) and the resulting supernatant transferred to a clean centrifuge tube. The solution was washed twice with diethyl ether, the organic phase discarded, and residual ether and acetone removed with a stream of nitrogen. An equal volume of 1 M Tris(hydroxymethyl)methylaminopropanesulfonic acid buffer (pH 9.2) and 2 vol of 1 mM dipicrylamine in methylene dichloride were added to the aqueous residue. After mixing and centrifuging, the aqueous phase was discarded and the organic phase was transferred to a clean centrifuge tube and evaporated to dryness with nitrogen. Silver *p*-toluenesulfonate (5 mM in acetonitrile, 0.5 ml) and 50 μl of propionyl chloride were added, mixed, allowed to react at room temperature for 5 min, and evaporated to dryness. Sodium benzenethiolate in anhydrous butanone was added and the sample incubated at 80° for 45 min to demethylate the quaternary amines. This was followed by two liquid partition steps to separate tertiary amines from neutral and acidic compounds and an aliquot of the residue was injected into a Hewlett-Packard model 5840 gas chromatograph coupled to a Hewlett-Packard model 5985A quadrapole mass spectrometer. Compounds were fragmented by electron impact ionization and the system was run in the single ion detection mode to maximize quantitation sensitivity for unlabeled acetylcholine at $m/e = 58$, tracer [$^2H_4$]acetylcholine at $m/e = 60$, and internal standard at $m/e = 64$.

Choline uptake. High affinity choline transport was measured in crude synaptosomal fractions (P_2) using [^{3}H]choline. Cortices were gently homogenized in 20 vol of 0.32 M sucrose and centrifuged at 1,000 × *g* for 10 min to sediment debris and nuclei. The supernatant was centrifuged at 12,000 × *g* for 20 min to obtain the P_2 fraction. The tissue preparation was suspended in Krebs-phosphate buffer and incubated with [^{3}H]choline (1.0 μM or 0.25–3.0 μM for kinetic studies). High affinity transport of choline was measured as the difference between the transport at 37° and 0° during a 3-min incubation with 0.1–0.2 mg of protein. The transport process was stopped by rapid centrifugation, the pellets washed with buffer, dissolved in base, and the transported [^{3}H]choline counted.

P_3 and S_3 preparation. Brain slices were gently homogenized in Krebs-phosphate buffer and the P_2 fraction prepared as described above. The pellet was resuspended in H_2O (2 ml) containing paraoxon (20 μM) and centrifuged at 100,000 × *g* for 60 min. Acetylcholine was measured in the resulting supernatant (S_3; labile bound or cytoplasmic fraction) and pellet (P_3; stable bound or vesicular fraction). Acetylcholine synthesis and distribution in these fractions have been described in detail previously (13).

Results

Acetylcholine synthesis and release. Cortical brain slices were used to study the effects of AH5183 and quinacrine on acetylcholine metabolism because the release of acetylcholine from slices displays a large response (approximately 8-fold) to K^+ depolarization (Table 1) as compared to the small response (1.5- to 2-fold) seen in synaptosomal preparations (14). The effects of varying concentrations of AH5183 and quinacrine on the content and release of acetylcholine from cortical slices incubated in high K^+ are shown in Fig. 1, A and B, respectively. AH5183 was a potent inhibitor of acetylcholine release, causing

TABLE 1
Cortical slice content and release of acetylcholine

Slices were prepared and incubated in buffer containing 10 μM [$^2H_4$] choline, 20 μM paraoxon, and low K^+ (4.9 mM) or high K^+ (50 mM) as described in Methods. Acetylcholine was determined by GCMS. Values are means ± SE ($n = 15$).

	Acetylcholine	[$^2H_4$]Acetylcholine	Total acetylcholine
	pmol/mg protein		
Slice			
Low K^+	470 ± 21	89 ± 9	559 ± 23
High K^+	179 ± 10	108 ± 8	287 ± 19
Released			
Low K^+	47 ± 3	28 ± 2	75 ± 5
High K^+	428 ± 16	202 ± 18	630 ± 33
Total			
Low K^+	517 ± 19	117 ± 8	634 ± 20
High K^+	607 ± 13	310 ± 14	917 ± 27

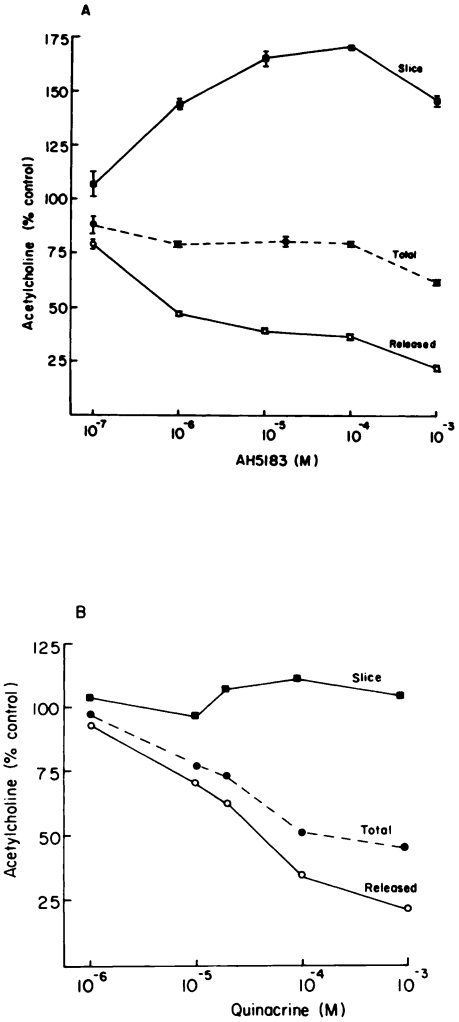

Fig. 1. Effects of (A) AH5183 and (B) quinacrine on acetylcholine levels and release in rat cortical slices. Slices of rat cortex were prepared and incubated, as described in Methods, for 15 min at 37° in the presence of 20 μM paraoxon (to inhibit acetylcholinesterase), 50 mM KCl, and the concentration of inhibitor indicated. The acetylcholine released and in the slices was measured by GCMS. Values are given as percentage of control levels which are presented in Table 1 and are means of three to four experiments for AH5183 (± SE) and of two for quinacrine, each carried out in duplicate or triplicate. Total indicates the sum of the tissue and released acetylcholine.

a 50% inhibition at a concentration of 1 μM. As the inhibition of acetylcholine release became greater with increasing concentrations of AH5183, there was a concomitant increase in the level of acetylcholine in the slices. Increased concentrations of acetylcholine in the slices do not indicate increased synthesis of acetylcholine but actually indicate less depletion caused by K^+-stimulated release of acetylcholine (see Table 1). These combined effects resulted in only a 10–20% decrease in the total amount (slice plus released) of acetylcholine except at the highest concentration of AH5183. It is the total amount of acetylcholine present in the incubation (not only the concentration of acetylcholine in the slice) that indicates effects of inhibitors on the synthesis of acetylcholine. These results imply a major effect by AH5183 on acetylcholine release and a lesser effect on acetylcholine synthesis.

Quinacrine also inhibited the K^+-stimulated release of acetylcholine, but it was about 50-fold less potent than AH5183 as release was inhibited by 50% by 50 μM quinacrine (Fig. 1B). In contrast to the effects of AH5183, there was no increase in the concentration of acetylcholine in the slices and there was a concentration-dependent decrease in the total amount of acetylcholine present indicating that both synthesis and release of acetylcholine are inhibited by quinacrine.

Slices were incubated with [$^2H_4$]choline to quantitate the rate of synthesis of newly synthesized [$^2H_4$]acetylcholine and to distinguish its release and subcellular compartmentation from that of endogenous acetylcholine. AH5183 inhibited the release of both unlabeled (Fig. 2A) and labeled (Fig. 2B) acetylcholine, but the effect was clearly greater on the latter. AH5183 increased the concentrations (i.e., reduced K^+-stimulated depletion) of both forms of acetylcholine in the slices but the increase of unlabeled acetylcholine was much greater than that of newly synthesized acetylcholine. The total amount of unlabeled acetylcholine was unchanged with increasing concentrations of AH5183 but there was a concentration-dependent decline in the total amount of labeled acetylcholine. Thus, although AH5183 had a greater effect on release, the synthesis of acetylcholine was also inhibited. This was not due to direct inhibition of choline acetyltransferase (0.1 and 1 mM AH5183 had no effect on the activity of partially purified choline acetyltransferase).[1]

The effects of quinacrine differed in several ways from those of AH5183. Quinacrine inhibited the K^+-stimulated release of unlabeled (Fig. 3A) and labeled (Fig. 3B) acetylcholine approximately equally up to 100 μM quinacrine, at which point its effect was greater on the newly synthesized acetylcholine. In contrast to the effects of AH5183, there was no increase in the concentration of newly synthesized acetylcholine in the slices and endogenous acetylcholine only increased in the slices at the higher concentrations of quinacrine. The total amounts of both labeled and unlabeled acetylcholine were reduced by quinacrine in a dose-dependent manner. Thus, quinacrine is a weaker inhibitor of the K^+-stimulated acetylcholine release than is AH5183, but it is a more potent inhibitor of acetylcholine synthesis. Quinacrine is also a weak inhibitor of choline acetyltransferase but it is unlikely that this played a role in the present experiments (0.1 mM and 1 mM quinacrine inhibited partially purified choline acetyltransferase by 8% and 20%, respectively).[1]

Choline transport. High affinity choline transport is the rate-limiting step in the synthesis of acetylcholine (15). The effects of AH5183 and quinacrine were measured on high affinity choline uptake in cortical synaptosomes to test for direct effects on acetylcholine synthesis, as well as to determine the specificities of their sites of action. Fig. 4 shows that both AH5183 and quinacrine were effective inhibitors of high affinity choline transport, with IC_{50} concentrations of 25 μM and 6 μM, respectively. This is in agreement with the results obtained using brain slices which indicated that quinacrine is a more potent inhibitor of acetylcholine synthesis than is AH5183.

AH5183 had a biphasic inhibitory effect on choline transport, producing approximately 15% inhibition between 0.5 and 5 μM, followed by a second, major inhibitory effect at higher concentrations. The effect of AH5183 was further characterized by determining its effect (using 50 μM) on the V_{max} and apparent K_T for choline (Fig. 5). These data indicate a competitive-like inhibition of choline transport by AH5183 because the V_{max} (22.7 versus 25.6 pmol/min/mg of protein) was not significantly altered while the apparent K_T for choline was increased from 1.6 to 5.4 μM. The K_i was determined to be 13 μM.

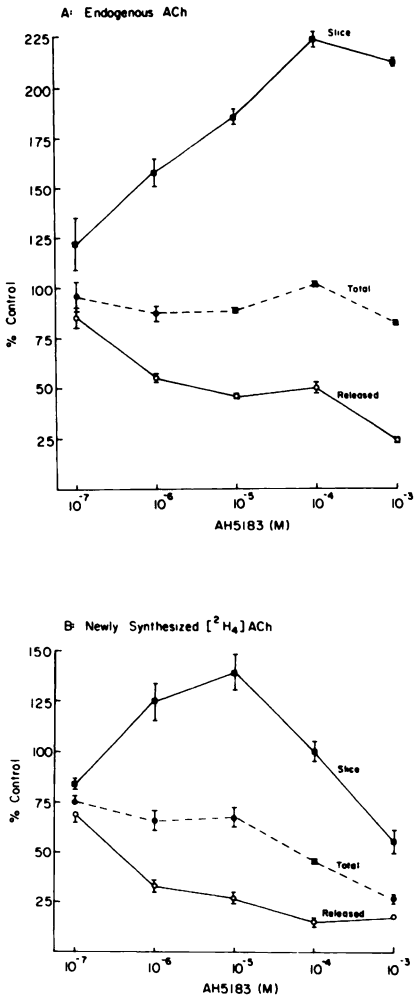

Fig. 2. Effects of AH5183 on (A) endogenous, unlabeled acetylcholine (*ACh*) and (B) newly synthesized [$^2H_4$] acetylcholine in rat cortical slices. Slices of rat cortex were prepared and incubated, as described in Methods, for 15 min at 37° in the presence of 20 μM paraoxon, 50 mM KCl, 10 μM [$^2H_4$]choline (as a tracer to label newly synthesized acetylcholine), and the concentration of AH5183 indicated. The acetylcholine released and in the slices was measured by GCMS. Values are means ± SE of three to four experiments, each carried out in duplicate or triplicate.

[1] G. V. W. Johnson, unpublished results.

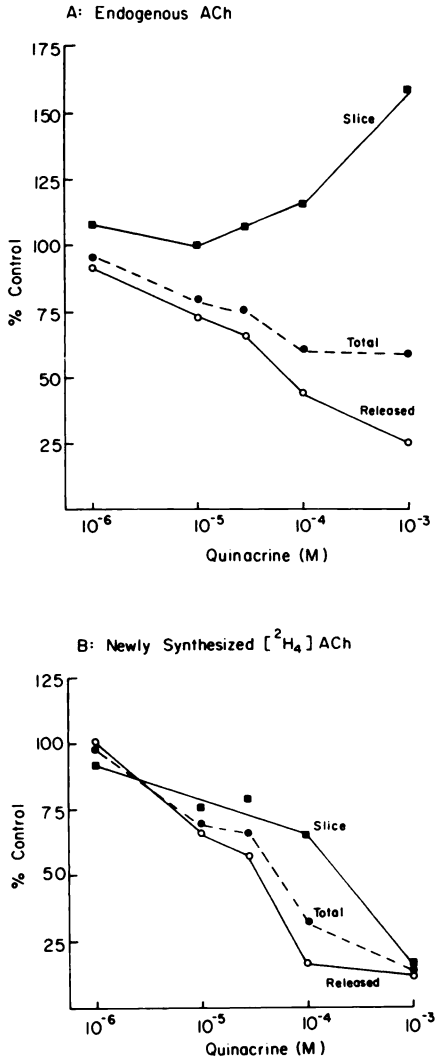

Fig. 3. Effects of quinacrine on (A) endogenous, unlabeled acetylcholine (*ACh*) and (B) newly synthesized [$^2H_4$]acetylcholine in rat cortical slices. Slices of rat cortex were prepared and incubated, as described in Methods, for 15 min at 37° in the presence of 20 μM paraoxon, 50 mM KCl, 10 μM [$^2H_4$]choline (as a tracer to label newly synthesized acetylcholine), and the indicated concentration of quinacrine. The acetylcholine released and in the slices was measured by GCMS. Values are means of two experiments, each carried out in duplicate or triplicate.

Acetylcholine compartmentation. The final goal of this project was to determine whether AH5183 alters the subcellular localization of acetylcholine in brain tissue. (Quinacrine was not studied because inhibition of acetylcholine release appeared to be due primarily to inhibition of acetylcholine synthesis.) Brain slices were incubated, as described above, with 100 μM AH5183. The concentrations of endogenous and newly synthesized acetylcholine were measured in duplicate samples of slices and in parallel samples in which the P_3 and S_3 fractions were prepared. In cortical slices incubated in high K^+, AH5183 reduced the total amount of acetylcholine released by 61%, with a greater effect on newly synthesized than on endogenous acetylcholine (Fig. 6A). AH5183 also caused increased tissue concentrations of acetylcholine (i.e., reduced the depletion of acetylcholine due to the K^+-stimulated release), with the concentration of endogenous acetylcholine increased to a greater extent than newly synthesized acetylcholine. Examination of the P_3 and S_3 fractions revealed a clear effect of AH5183; the acetylcholine concentration in the S_3 fraction was increased by almost 2-fold while the acetylcholine concentration in the P_3 fraction was not altered from control levels. As shown in Table 1, 68% of the acetylcholine released by high K^+ is unlabeled acetylcholine in control slices (i.e., 428/630) and in the presence of AH5183 71% of the acetylcholine accumulated over control levels in the S_3 fraction is also unlabeled acetylcholine, supporting a direct relationship between these two events. It is important to note that the total amount of newly synthesized acetylcholine present (tissue plus released) is decreased by 10^{-4} M AH5183 (see Fig. 2B) even though the amount in the S_3 fraction is increased. This indicates that the reduction in the released labeled acetylcholine alone (rather than tissue concentrations) accounts for the decreased amount of labeled acetylcholine synthesized, indicating that the primary site of action of AH5183 is on the release process rather than the synthetic process.

The turnover rate of acetylcholine is very rapid in K^+-

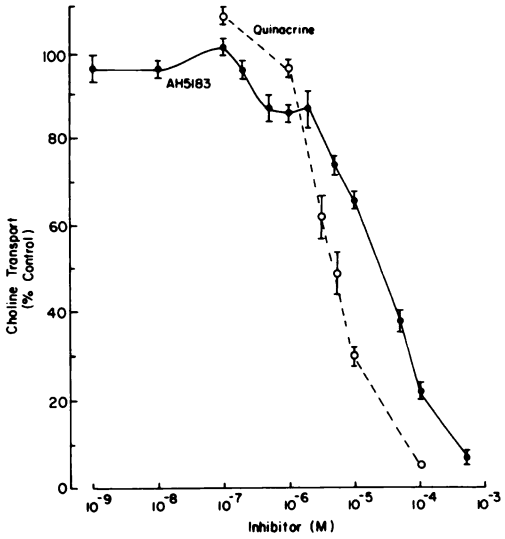

Fig. 4. Effects of AH5183 and quinacrine on high affinity choline transport. Cortical synaptosomes were incubated with 1 μM [^3H]choline for 3 min at 37° or 0° in the presence of varying concentrations of AH5183 (●) or quinacrine (○). Choline transport was quantitated as described in Methods. Values are means ± SE of three to five experiments carried out in triplicate. Control rates were 9.5 ± 0.9 pmol/min/mg of protein.

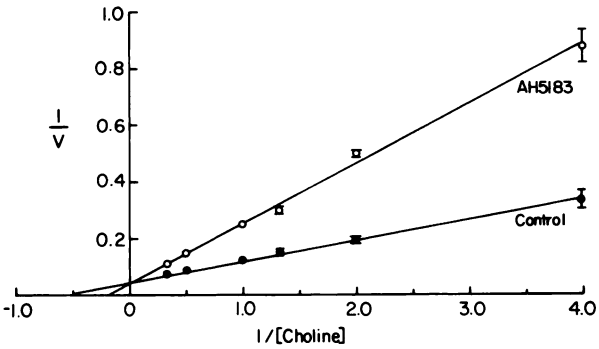

Fig. 5. Effect of AH5183 on the kinetics of high affinity choline transport. Cortical synaptosomes were incubated with 50 μM AH5183 and 0.25–3.0 μM [^3H]choline for 3 min at 37° or 0°. Values are means ± SE of four experiments carried out in triplicate. *V* = picomoles/min/mg of protein.

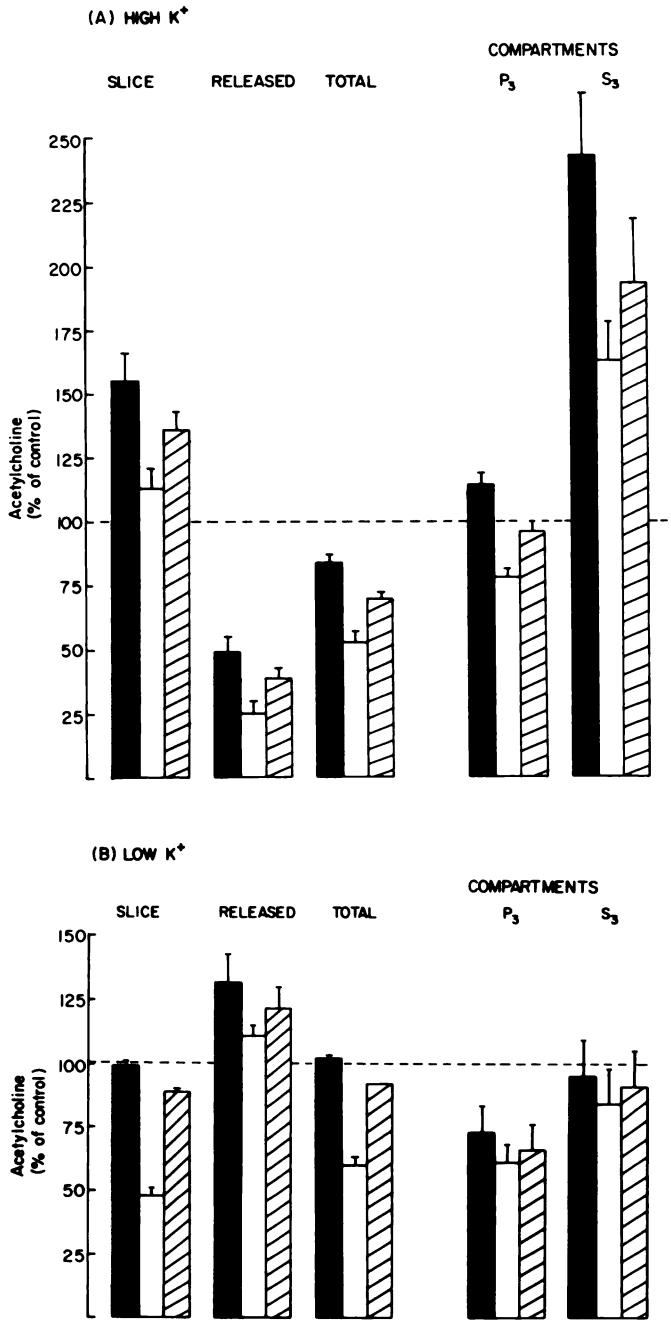

Fig. 6. Cortical slice acetylcholine concentration, release, and subcellular distribution following incubation with 100 μM AH5183 in (A) high K^+ or (B) low K^+. Tissue was treated as described in Methods. Values are means ± SE of three experiments, each carried out in duplicate. ■, endogenous acetylcholine; □, newly synthesized acetylcholine; ▨, total acetylcholine.

depolarized tissue. As indicated in Table 1, high K^+ releases more than the total slice acetylcholine content present in slices incubated in low K^+ (i.e., 630 compared to 559 pmol/mg of protein). To examine the effects of AH5183 on acetylcholine compartmentation without this rapid turnover rate, we measured the effects of AH5183 on acetylcholine when slices were incubated in low K^+ (Fig. 6B). AH5183 (100 μM) reduced the synthesis of labeled acetylcholine by 40% and increased the amount of acetylcholine released by 22%. The concentration of acetylcholine in the P_3 fraction was reduced by 34% while there was no change from controls in the S_3 fraction. Therefore, under conditions where the vesicles were not depleted it was possible to show an AH5183-dependent blockade of acetylcholine loading.

Discussion

The effects of AH5183 and quinacrine on brain acetylcholine metabolism were studied because these compounds were reported to be the two most potent inhibitors of acetylcholine transport into vesicles isolated from the *Torpedo* electric organ (1). The present study has shown that both compounds inhibit the K^+-stimulated release of acetylcholine from rat cortical slices. However, the mechanisms responsible for this effect appear to be different for the two drugs; the inhibition of acetylcholine release caused by quinacrine seems to be primarily a result of inhibition of acetylcholine synthesis while AH5183 seems to act at a site more directly associated with the release process. For this reason, as well as the fact that AH5183 was 50-fold more potent than quinacrine in reducing acetylcholine release and is a 10-fold more potent inhibitor of vesicular acetylcholine loading (1), the effects of AH5183 on brain acetylcholine metabolism were studied in greater detail.

The subcellular distribution of acetylcholine in brain slices was measured in the presence of AH5183 at a concentration (100 μM) that inhibited K^+-stimulated acetylcholine release by 60%. In low K^+, AH5183 caused a 34% reduction in the P_3 acetylcholine content but did not alter the acetylcholine concentration in the S_3 fraction. Since most vesicles are presumably filled under these conditions and since release is minimal, this result is consistent with the conclusion that AH5183 inhibits vesicular acetylcholine loading in brain slices and that only a small fraction of the vesicles are taking up acetylcholine under resting conditions. Clearly, however, AH5183 is less effective in impairing vesicle acetylcholine loading in brain slices than in isolated *Torpedo* vesicles since the Ic_{50} for the latter is 40 nM, whereas 100 μM AH5183 only blocked the loading of newly synthesized acetylcholine (in low K^+) by 40%. Diffusion barriers in the brain slices might account for the higher concentration of AH5183 required in slices compared to purified vesicles.

The effects of AH5183 on the subcellular distribution of acetylcholine in slices incubated in high K^+ were more intriguing. Under these conditions, the concentration of acetylcholine in S_3 was doubled by AH5183 but the P_3 acetylcholine content was not altered. Since these fractions were obtained from slices incubated in high K^+, the acetylcholine content was depleted by 50% (Table 1) so that the acetylcholine measured in the P_3 fraction consists of relatively inactive vesicular acetylcholine. Therefore, it is not surprising that AH5183 did not alter the acetylcholine concentration in this fraction compared to controls.

The observed effects of AH5183 on acetylcholine metabolism in cortical slices are summarized in Fig. 7. Considering these results, two hypotheses can be proposed to explain the effects of AH5183. First, based on the reported inhibitory effect of AH5183 on vesicular acetylcholine loading (1) and the present results, in brain slices AH5183 may inhibit the loading of acetylcholine into a very active and labile subpopulation of vesicles that is the source of the majority of the acetylcholine released by K^+-depolarization. Barker et al. (16) demonstrated in guinea pig brain the existence of a small pool of vesicles that

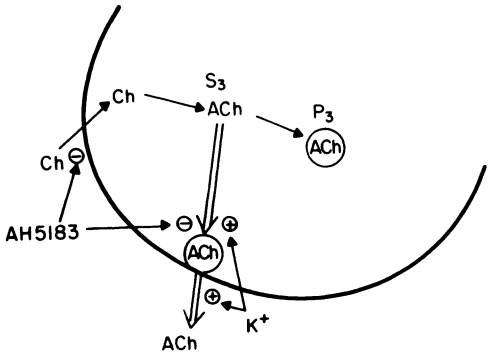

Fig. 7. Summary of the effects of AH5183 on acetylcholine (*ACh*) metabolism in cortical slices incubated in buffer containing 50 mM KCl.

were rapidly loaded with newly synthesized, labeled acetylcholine and a larger pool of vesicles containing a relatively stable concentration of primarily preformed acetylcholine. The rapidly labeled pool of vesicles was highly labile, was easily lost during tissue fractionation, and was suggested to be an active pool of vesicles located close to the synaptic membrane (16). In a series of studies, Zimmerman and coworkers also identified an active, highly labeled subfraction of vesicles in *Torpedo* electric organ (reviewed in Ref. 17). Therefore, the effects of AH5183 on acetylcholine metabolism reported here are consistent with this drug blocking the loading of acetylcholine into a small, active, labile subpopulation of vesicles which is the primary source of acetylcholine released by K^+ depolarization. Blockade by AH5183 of acetylcholine loading into these vesicles would then result in the observed blockade of acetylcholine release, the increased concentration of acetylcholine in the S_3 fraction, and no change in the acetylcholine concentration in the P_3 fraction, which consists of relatively inactive vesicles containing mostly preformed, unlabeled acetylcholine. This proposal is consistent with a recent study of choline analogs showing that the acetylated analog must be loaded into vesicles to be released (18).

The second hypothesis that can be proposed to explain the effects of AH5183 is based on the evidence that cytoplasmic acetylcholine may be the source of the released acetylcholine (reviewed in Ref. 19). If this is the case, AH5183 may inhibit acetylcholine binding to release sites at the synaptic membrane resulting in reduced release and the observed increase in acetylcholine content in the S_3 fraction. This hypothesis is not presently testable, but the effects of AH5183 on acetylcholine metabolism in slices incubated in low K^+ would seem to weaken its likelihood. In low K^+, AH5183 reduced the P_3 acetylcholine concentration by 34% (indicating an effect on vesicular loading) and increased the amount of acetylcholine released by 22%. We (15) and others (20, 21) have previously concluded that resting acetylcholine release from synaptosomes is derived from the cytoplasmic pool of acetylcholine. Consequently, in order for AH5183 to inhibit K^+-stimulated acetylcholine release, but not resting release, would require distinct cytoplasmic pools of acetylcholine or distinct release sites for resting and stimulated release if the second hypothesis of the mechanism of action of AH5183 is tenable. Further experiments will be required to distinguish between these two possible mechanisms of action of AH5183.

Although the primary site of action of AH5183 is a site directly involved in the release of acetylcholine, AH5183 treatment also reduced the synthesis of acetylcholine. In brain slice we cannot deduce how much of the inhibition of acetylcholine synthesis was due to feedback inhibition resulting from impaired acetylcholine release and how much was due to a direct effect of AH5183 on the synthetic process. However, it is clear from the experiments using synaptosomes that AH5183 can inhibit high affinity choline transport, although this effect is weaker than its effect on acetylcholine release. At micromolar concentrations of AH5183, choline transport was inhibited in a competitive manner which is not surprising considering the structural similarity of AH5183 to choline. However, there was an intriguing biphasic effect of AH5183, with concentrations of AH5183 from 0.5 to 5 μM producing a plateau at 15% inhibition of choline transport. Although this is entirely speculative, it is possible that this small percentage of the choline transport system is directly coupled with acetylcholine synthesis and vesicular loading of acetylcholine as originally proposed by Barker and Mittag (22) and more recently studied by Nelson *et al.* (23).

Although quinacrine was the second most potent inhibitor of acetylcholine loading into purified vesicles from *Torpedo* reported by Anderson *et al.* (1), we found it to have marked differences from AH5183 on acetylcholine metabolism in brain slices. Quinacrine inhibited choline transport in synaptosomes and acetylcholine synthesis in brain slices. These inhibitory effects made it difficult to distinguish a direct effect of quinacrine on the release of acetylcholine. However, it would be interesting if these inhibitory effects of quinacrine on acetylcholine release could be related to other known effects of quinacrine, especially to its inhibition of phospholipase A_2 activity. Previous reports have linked inhibition of phospholipase A_2 by quinacrine to inhibition of neurotransmitter release (9–11). Additionally, vesicles contain a phospholipase A_2 enzyme and this has been suggested to play a role in exocytosis (24). Further studies of the effects of quinacrine may be useful to determine whether its effects on acetylcholine metabolism and phospholipase A_2 activity are directly associated. It may also be useful to determine whether or not AH5183 inhibits vesicular phospholipase A_2 activity.

In summary, both AH5183 and quinacrine were shown to be effective inhibitors of the K^+-stimulated release of acetylcholine from rat cortical slices. This effect of AH5183 appears to be a direct consequence of its inhibition of the vesicular loading of acetylcholine although it also possesses acetylcholine synthesis inhibitory properties. However, the inhibition of acetylcholine release by quinacrine appears to be primarily a consequence of inhibition of acetylcholine synthesis.

Acknowledgments

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