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ACETYLCHOLINE RELEASING AGENTS AS COGNITION ACTIVATORS. CHEMISTRY AND PHARMACOLOGY OF A SERIES OF UREAS

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Abstract: We have sought to improve upon the activity of the well known AcCh-releasing agents, 4-aminopyridine and 3,4-diaminopyridine. This work has led to the discovery of a series of ureas with AcCh-releasing properties. From this series, compound 5 has emerged as a potent AcCh-releasing agent with promising *in vivo* activity.

The cholinergic hypothesis of age-related dementia has provided a catalyst for exploring various means of enhancing cholinergic function[1, 2, 3]. Focusing on the cholinergic synapse, one might consider new therapeutic agents that target the pre-[4], intra-[5] and/or post-junctional sites[6]. Cholinesterase inhibitors and muscarinic agonists are representative of the latter two categories. Agents that act at the pre-synaptic cholinergic neuron are much less well known and characterized. Among the best studied examples to date are the aminopyridines, which increase the release of AcCh in a variety of brain regions, owing to effects on K^+ and Ca^{2+} ion channels[7-9]. Recently a new series of AcCh releasing agents was disclosed related to DUP 996; these compounds are also claimed to induce AcCh release[10], though the mechanism of action remains unclear. In principle, stimulated AcCh release could provide a more natural phasic enhancement of cholinergic function than persistent tonic post-synaptic stimulation.

4-Aminopyridine and 3,4-diaminopyridine cause a Ca^{2+} dependent increase in the release of AcCh in a variety of brain regions. Table 1 shows the effects of 4-aminopyridine and 3,4-diaminopyridine on three brain regions at four concentrations[11,12]. The effects on release are not brain region specific, though the greatest effect appears to be in the striatum. Increases in AcCh release were observed in all three regions tested, with the most pronounced effect on basal release. 3,4-Diaminopyridine was more potent than 4-aminopyridine in the striatum.

Table 1

Acetylcholine Release, % Control

		4-Aminopyridine		3,4-Diaminopyridine	
Brain Region	Concentration	Basal	K+-Stim	Basal	K+-Stim
Hippocampus	1 μM	112.7	97.3	106.2	121.9**
	10 μM	116.8*	96.5	114.0**	100.8
	50 μM	- - -	- - -	119.0**	119.6*
	100 μM	128 1**	105.0	127.8**	106.9
Cortex	1 μM	96.2	96.7	104.2	93.5
	10 μM	102.4	104.0	113.8**	108.1
	50 μM	112.2	99.9	116.0**	103.0
	100μM	118.0*	96.3	120.8**	110.8
Striatum	1 μM	107.6	94.0	129.3**	88.8
	10 μM	131.5	105.5	164.2**	111.5
	50 μM	- - -	- - -	180.9**	118.5*
	100 μM	170.9**	131.6*	224.8**	105.9

*p<0.05, **p<0.01

A substructure search of our proprietary chemical files uncovered a 4-pyridinyl urea related to 4-aminopyridine, specifically compound **3** (Tables 2 and 3). In vitro, compound **3** caused a profound and selective increase in both basal and K+ stimulated AcCh release in the striatum. 3,4-Diaminopyridine, on the other hand, caused an increase only in basal AcCh release in the striatum.

A wide variety of ureas were subsequently synthesized and found to have a diverse range of effects on AcCh release in the hippocampus (see Table 2).

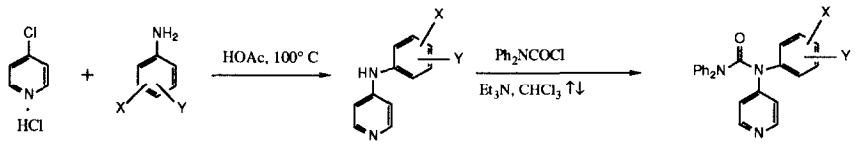
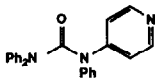
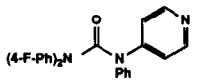
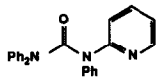
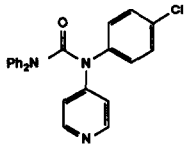
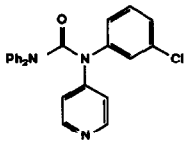
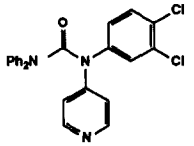
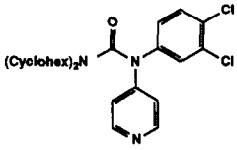
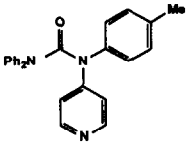


Table 2

Compound	Structure	Acetylcholine Release 100μM, % Control (Hippocampus) Basal	K^+ Stim.	Mouse Water Maze MED† mg/kg
3		95.4	129.8	
4		115.6	48.9**	
5		113.5	318 3**	≤1.0
6		159.2**	231.4**	
7		103.4	9.8**	
8		124.9**	46.6**	32
9		335.2**	233.2**	
10		186.4**	229**	>32.0

*p < 0.05, **p < 0.01

† minimum effective oral dose

Although the aminopyridines show their greatest effect in the striatum, we examined the acetylcholine releasing ability of our compounds in the hippocampus, since it is the area most affected by Alzheimer's disease[1]. The range of activity from these compounds encompassed not only the desired profile of no change in basal and increase in K^+-stimulated release, but also included compounds such as **7** (which has no effect on basal but decreased K^+-stimulated release), compound **8** (which increased basal but decreased K^+-stimulated release), and compound **9** (which increased both basal and K^+-stimulated release). The mechanism of action is unlikely to be the same as that of the aminopyridines since their release of AcCh is not K^+ dependent. Upon examining the SAR of these compounds no coherent pattern of activity emerged, as small changes in structure resulted in significant changes in activity (e.g., compounds **3**, **4**, **6**, **7**, and **8**).These effects were also seen with simple phenyl ureas (see Table 3, compounds **11-14**). In this limited series of phenyl ureas, increased AcCh releasing ability correlated with increasing lipophilicity. This correlation holds only for basal release and breaks down for the series as a whole.

A number of these compounds also improved spatial memory in hippocampally deficient mice (see Table 2 and Figure 1)[13,14]. The test is a modified Morris water maze in which hippocampally deficient C57BL/10 mice were tested for latency to reach a hidden platform. Activity in this paradigm, however, does not correlate with AcCh-releasing ability, and may not be associated with it. The lack of toxicity of these compounds in repeated dosing studies suggests that they do not deplete stores of AcCh.

There are a number of methods one could envision for modulating acetylcholine release, either through the presynaptic muscarinic receptor or through a number of other neurotransmitter systems, e.g., dopamine, glutamate, serotonin[15]. The AcCh releasing compounds show little or no activity at a wide variety of known CNS receptors and enzyme systems (e.g., α_1, α_2, β_1, β_2, D_1, D_2, A_1, A_2; Schwarz *et al.*, unpublished observations), and the exact mechanism of action responsible for the AcCh release enhancing properties remains unknown.

Clearly the most interesting of the compounds examined is compound **5**. It shows little if any basal release of acetylcholine in the hippocampus while evoking the largest K^+ stimulated release of acetylcholine that we have observed. Compound **5** also causes K^+ stimulated release in all three brain regions examined with the largest increase residing in the hippocampus. While the exact mechanism through which the acetylcholine release is elicited remains unclear, it is clear that this compound has positive cognitive effects. This compound is currently undergoing further evaluation.

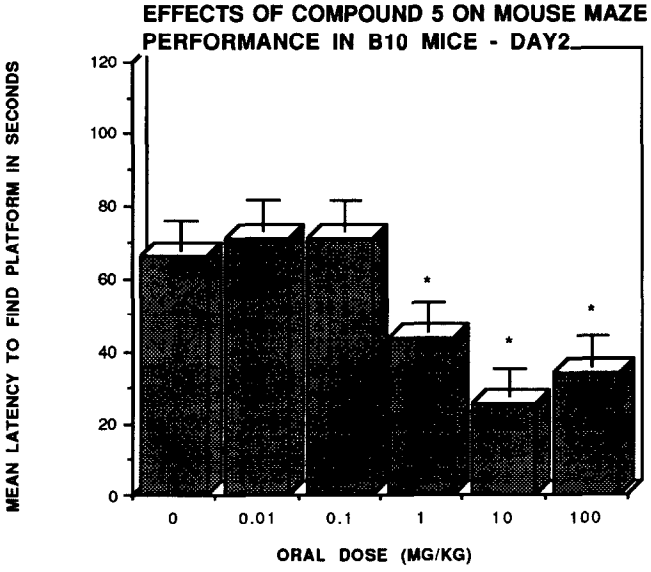
Table 3

Acetylcholine Release, 100 μM, % Control

Compound	Cortex		Hippocampus		Striatum	
	Basal	K^+-Stim	Basal	K^+-Stim	Basal	K^+-Stim
$PhHNCONH_2$ (11)	105.8	116.4	94.1	107.9	99.3	99.8
Ph_2NCONH_2 (12)	105.1	111.4	92.2	128.7**	105.9	98.9
PhHNCONHPh (13)	101.9	125.8*	89.0	138.6**	97.1	98.8
$Ph_2NCONHPh$ (14)	100.3	195.7**	78.0*	151.3**	259.5**	224.0**
$Ph_2NCONPh_2$ (15)	144.1**	209.3**	137.8**	420.6**	402.4**	303.7**
3	95.2	100.0	95.4	129.8	279.8**	206.5**
5	130.0**	158.8**	113.5	318.3**	232.1**	266.6**

*$p < 0.05$, **$p < 0.01$

Figure 1



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12. Slices from rat striatum, cortex, or hippocampus were incubated with ^{3}H-choline (0.01 μM) for 15 min. at 37°C in Krebs-Ringer Hepes buffered media, pH 7.2. The slices were washed with media (5mL, 3x) and incubated an additional 15 min. at 37°C in normal media or media with elevated K+ in the presence or absence of test compound. The reaction was terminated and centrifuged and the amount of radioactivity was measured in both fractions by liquid scintillation counting.
    Results were calculated as follows:
    Basal % Control = (% Total drug, basal/% Total control,basal)X 100%.

$$\text{K+-Stim. \% Control} = \frac{(\%\ \text{Total}_{\text{drug, K+-Stim}} - \%\ \text{Total}_{\text{control, basal}})}{(\%\ \text{Total}_{\text{control, K+-Stim.}} - \%\ \text{Total}_{\text{control, basal}})} \times 100$$

    % Total = $\text{cpm}_{\text{medium}} / (\text{cpm}_{\text{medium}} + \text{cpm}_{\text{tissue}}) \times 100\%$
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