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Cholinergic Function and Memory: Extensive Inhibition of Choline Acetyltransferase Fails to Impair Radial Maze Performance in Rats

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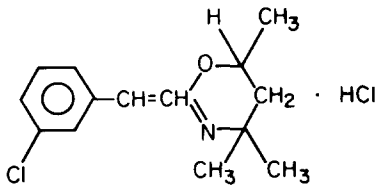
WENK, G., J. SWEENEY, D. HUGHEY, J. CARSON AND D. OLTON. *Cholinergic function and memory: Extensive inhibition of choline acetyltransferase fails to impair radial maze performance in rats.* PHARMACOL BIOCHEM BEHAV **25**(3) 521–526, 1986.—The present study investigated the effects of a potent inhibitor of choline acetyltransferase (ChAT), BW813U, on the choice accuracy of rats in the radial arm maze. BW813U (100 mg/kg, IP) produced a rapid (within 1 hour) and substantial decrease in ChAT activity throughout the brain, ranging from 66% (hippocampus) to 80% (caudate nucleus) that lasted up to 5 days. A single injection (50 mg/kg, IP) into rats with lesions (using ibotenic acid) in the nucleus basalis magnocellularis and medial septal area, decreased ChAT activity by 75% and 60% in the cortex and hippocampus, respectively. Lesioned and unlesioned rats were trained on the radial arm maze until they reached a criterion level of performance. Each rat then received an injection of BW813U (50 or 100 mg/kg, IP). Choice accuracy was not impaired at any time following the injection. The lack of effect on performance may be due to 2 possible factors: The radial maze retention paradigm chosen may not be sufficiently difficult, or the decrease in acetylcholine production was not sufficient to affect behavior. Compensation by non-cholinergic neural systems might account for the insensitivity of the rats to significant cholinergic depletion.

Choline acetyltransferase inhibition Radial maze Spatial memory Rats Acetylcholine BW813U

THE acetylcholinergic system has been implicated in memory and learning [6, 7, 15, 46]. Pharmacological agents that affect cholinergic activity alter memory [5, 16, 37, 39, 47, 51]. The influence of injury to the cholinergic cells in the nucleus basalis magnocellularis (NBM) or the medial septal area (MSA), that project to the cortex and hippocampus, respectively [25, 26, 29, 40, 44, 45], on memory has been evaluated in many different behavioral tasks [1, 17, 22, 23, 27, 31, 38, 43]. Neocortical and hippocampal levels of the cholinergic anabolic enzyme choline acetyltransferase (ChAT) assessed the effectiveness of lesions produced by injections of ibotenic acid (IBO) [12,52]. Decreased ChAT activity in these regions correlated with impaired performance of rats in tasks that require learning and memory. In humans, reduced regional levels of ChAT activity correlates with memory impairments and dementia associated with aging [7] and Alzheimer's disease [13, 41, 42, 48] as well as with the loss of basal forebrain cells [3, 56, 57]. However, decreased ChAT activity does not occur independently of damage elsewhere in the brain [48,56]. Many non-cholinergic neurotransmitter systems are affected as well [8, 19, 20, 33, 35]. Consequently, one cannot tell if the ChAT changes alone are causally related to the memory impairments.

Styrlpyridines, particularly quaternary salts, e.g., N-methyl-4-(I-naphthylvinyl)pyridinium iodide, NVP [54], have been reported as potent, selective inhibitors of ChAT, [11,54]. A major limitation has been the quaternary ammonium characteristic, which rendered the compounds incapable of penetration through the blood-brain barrier. Replacement of the purine moiety by oxazolines and oxazines has afforded an effective ChAT inhibitor capable of crossing the blood-brain barrier. These agents enable behavioral studies in animals after a single intraperitoneal injection. A styryloxazine, BW813U (Burroughs-Wellcome), was one of the most potent of the compounds investigated that also did not have a significant effect on acetylcholinesterase or monoamine oxidase activity [36] (Fig. 1).

The present study used BW813U to produce a discrete and substantial reduction in ChAT activity, of a magnitude larger than typically seen in Alzheimer's disease [42,48] or following the production of NBM lesions [22]. If the memory impairments in Alzheimer's disease and after NBM lesions are due directly to the decrease in ChAT activity and the subsequent decrease in acetylcholine production, then similar impairments should be seen here. If, however, those memory impairments are due to the loss of non-cholinergic



BW 813U

FIG. 1. Structure of BW813U, a non-quaternary, water-soluble, irreversible inhibitor of ChAT activity.

systems as well, then a selective inhibition of ChAT activity should have no effect.

Rats with and without basal forebrain injury were trained on the radial maze, injected with BW813U to selectively inhibit brain ChAT activity, and then tested further on the radial maze. The radial maze task was chosen because choice accuracy can be impaired by cholinergic deafferentation or anti-cholinergic drugs [2, 23, 24, 30].

METHOD

Subjects

The subjects were 80 male Sprague-Dawley rats obtained from Charles River Breeding Laboratories. They weighed between 325 and 375 g at the start of testing and were housed in pairs in standard rodent cages, 24×40×18 cm, with free access to water, and a 16 hour/8 hour light/dark cycle with lights on at 0700. Each rat in Experiments 4 and 5 was deprived to 85% of its ad lib. weight prior to behavioral training and was maintained at this weight, plus 5 g per week for growth, throughout testing. At the completion of the day's testing, each rat was given the appropriate amount of Charles River Rat Formula.

Experimental Design

Biochemical studies. Experiment 1: Twenty-five rats were injected with BW813U (50 mg/kg, IP) and sacrificed at various times afterwards.

Experiment 2: Twenty-one rats were injected with BW813U (25, 50, or 100 mg/kg, IP) and sacrificed 1 hour later.

Experiment 3: Four rats received 2 μl of BW813U (100 μM) injected under the dura over the frontal lobes, bilaterally, and were sacrificed 1 week later.

Behavioral studies. Experiment 4: Fourteen naive rats were trained on the radial maze until they achieved a criterion level of performance. Rats that were ultimately used in the high dose (100 mg/kg) group began training 1 week after the low dose (50 mg/kg) group. Each rat then received an injection of BW813U (initially, 50 or 100 mg/kg, IP) and continued testing on the radial maze. To further deplete axonal terminal acetylcholine stores, each rat was given (1) a supplemental injection of BW813U (100 mg/kg, IP) on testing Days 27 and 35, and (2) a single injection of pentylenetetrazol on Day 24 (75 mg/kg, IP) to stimulate acetylcholine release [4]. All rats were sacrificed on Day 42.

Experiment 5: Sixteen rats received lesions in the NBM and MSA and 9 received sham operations. Ten days after surgery, 5 lesioned rats received a single injection of BW813U (50 mg/kg, IP) and were sacrificed 1 hour later. The remaining rats in each group then began shaping on the 12-arm radial maze for 5 days. After 18 days of testing, each rat received a single injection of BW813U (100 mg/kg, IP). Behavioral testing continued for 10 days and then all rats were immediately sacrificed.

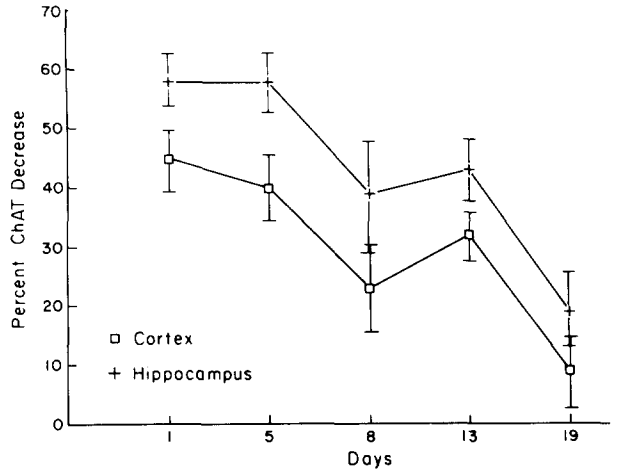


FIG. 2. Recovery of ChAT activity over time after a single injection of BW813U (50 mg/kg, IP). Each data point represents 5 rats.

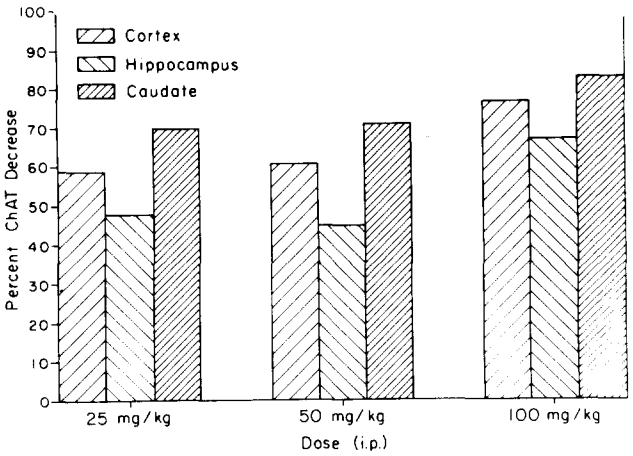


FIG. 3. Dose-response effects of BW813U (IP) on regional brain ChAT activity 1 hour after injection. Each data point represents 7 rats.

Surgery

Rats were pretreated with atropine methyl bromide (12.5 mg, Sigma Chemical Co.), anesthetized with Chloropent (Fort Dodge Laboratories, 3 cc/kg), and placed in a stereotaxic instrument. The scalp was incised and retracted. Lesions were produced by IBO which destroys cell bodies at the site of injection without damaging axons of passage [12]. The coordinates for the lesions were: NBM, 0.4 mm and 0.8 mm posterior to bregma, ±2.6 mm lateral to the midline, and 6.8 mm below the dura; MSA, 0.8 mm anterior to bregma, on the midline, and 5.8 mm below the dura. IBO (10 μg in 1 μl phosphate-buffered saline, pH 7.7) was infused via a 1.0 μl Hamilton microsyringe. The injected volume was either 0.6

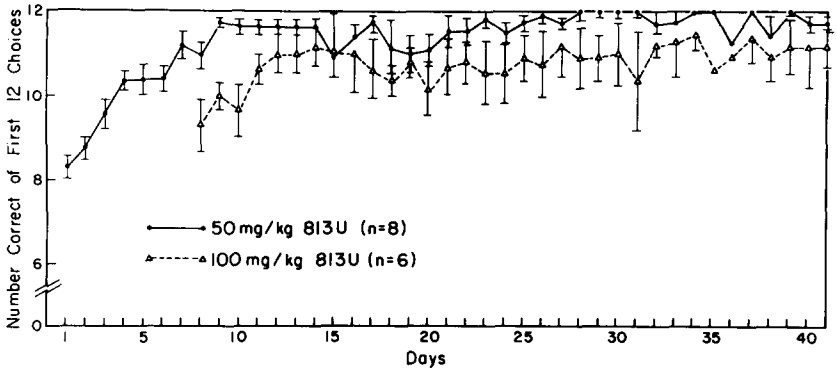


FIG. 4. Choice accuracy in the 12-arm radial maze (n=7). Each rat was given 50 mg/kg or 100 mg/kg (IP) of BW813U on Day 15. All rats were given 100 mg/kg on Days 27 and 35. Pentylenetetrazol (75 mg/kg, IP) was given to each rat on Day 24.

μl (MSA) or 0.4 μl (NBM). The needle was left in place for 5 minutes after infusion and then slowly withdrawn. The scalp was closed with sutures. The sham operations used the same procedure except no IBO was injected.

Behavioral Testing

The apparatus and procedures for shaping and testing rats on the 12-arm radial maze have been described previously [27]. Each rat was given one trial per day. Testing continued until: All 12 arms had been visited; the rat had made 20 responses; or 10 min had elapsed since the beginning of the trial. Criterion performance was achieved when each rat made 11 correct responses in the first 12 responses each day for 4 consecutive days.

Biochemistry

Each rat was decapitated and the brain was rapidly removed. Tissue samples (50–75 mg) were taken from the frontolateral neocortex, which did not include the cingulate area (areas 2 and 10 according to Krieg [28]), the dorsal hippocampus and the anterior dorsal caudate nucleus. ChAT activity was measured according to the method of Fonnum [18]. Protein was measured according to the method of Lowry *et al.* [32]. All assays were performed in triplicate.

Histology

After the removal of samples for biochemical analysis, the remaining brain tissue from rats in Experiment 5 was fixed in a 10% formalin:30% sucrose solution, frozen, and sectioned coronally at 30 μm with a frozen stage microtome. Sections throughout the lesion site were mounted on a glass slide and stained with cresyl violet. The size and location of the lesions were determined by microscopic examination for loss of magnocellular neurons and the presence of gliosis.

Statistics

Behavioral data were analyzed by a two-way (group × test session) ANOVA. Biochemical data were analyzed by Student's *t*-tests.

RESULTS

Biochemistry

Experiment 1: After a single injection (50 mg/kg, IP) ChAT activity was decreased ($p<0.01$) in the cortex and hippocampus by 58% and 45%, respectively, 1 day after the injection. ChAT activity then slowly recovered toward normal values during the next 19 days (Fig. 2).

Experiment 2: A single 25 mg/kg injection of BW813U significantly ($p<0.01$) decreased ChAT activity by 48% (hippocampus) to 69% (caudate) (Fig. 3). Increasing the dose to 100 mg/kg produced further decreases ($p<0.01$), ranging from 66% (hippocampus) to 80% (caudate).

Experiment 3: Subdural injections of 813U directed over the frontal cortex decreased ($p<0.01$) ChAT activity by 37% and 32%, in the anterior and posterior cortex, respectively, and by 33% in the hippocampus.

Experiment 4: BW813U treatment on Days 15, 27, and 35, decreased ($p<0.01$) ChAT activity in the frontal neocortex and hippocampus, respectively, by 61 and 50% for the low dose group (50 mg/kg) and by 63 and 40% for the high dose group (100 mg/kg).

Experiment 5: ChAT activity was significantly ($p<0.01$) decreased by 44% and 33% in the frontal cortex and hippocampus, respectively, by injections of IBO into the NBM and MSA. ChAT activity was significantly decreased ($p<0.01$) by 75% and 60% in the frontal cortex and hippocampus, respectively, 1 hour after a single BW813U (50 mg/kg) injection into lesioned rats.

BW813U treatment (100 mg/kg) decreased ($p<0.01$) cortical and hippocampal ChAT activity by 70 and 65%, respectively, in lesioned rats, and by 58 and 51%, respectively, in unlesioned rats tested on the radial maze.

Behavior

Experiment 4: All rats acheived criterion performance within 1 week after training began (Fig. 4). The low dose group began training earlier and continued until the high dose group reached criterion performance levels. Many rats showed atropine-like peripheral side-effects immediately following the BW813U treatment. Injections of BW813U (50 or 100 mg/kg, IP) and pentylenetetrazol did not impair choice

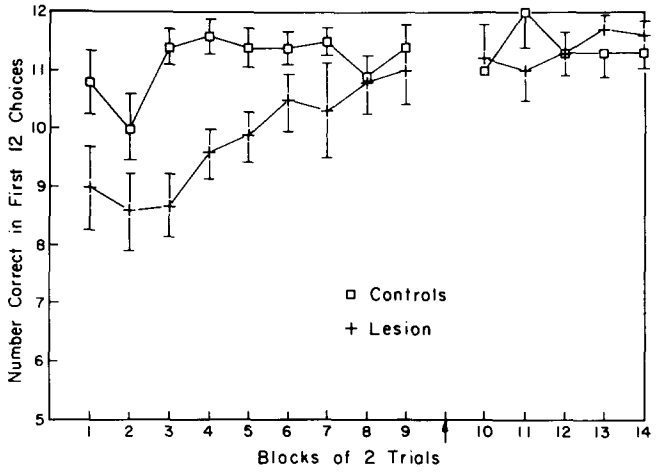


FIG. 5. Choice accuracy in the 12-arm radial maze of rats with NBM and MSA lesions and Controls before and after (arrow) treatment with BW813U (100 mg/kg, IP). *$p<0.05$, n=9.

accuracy ($F<1$), although the higher dose group tended to have slightly, but non-significantly, worse choice accuracy throughout testing. For 2 days after the third injection of BW813U on Day 35, all but 1 rat in each group stopped performing. However, on Day 37, all rats from both groups made at least twelve choices on each trial.

Experiment 5: Similar to Experiment 4, each unlesioned rat reached criterion performance levels by Day 8. The ANOVA showed that choice accuracy for both groups improved during testing, $F(1,322)=17.9$, $p<0.01$. A group by test session analysis and Sheffe contrasts revealed that the choice accuracy of rats with NBM and MSA lesions was significantly, $F(18,178)=26.9$, $p<0.05$, impaired relative to that of control rats for trials 1 through 10 inclusive (Fig. 5). All rats reached criterion performance by trial 18. BW813U injection did not significantly ($F<1$) affect the choice accuracy of lesioned or unlesioned rats. Only 1 rat in the control group and 6 rats from the lesioned group performed the task on the day of injection. However, all rats performed the next day.

Histology

Lesions in the NBM were centered in the substantia innominata and extended 2.5 mm caudally and 0.5 mm rostrally from the anterior commissure. Many of the cells in the horizontal limb of the diagonal band were destroyed by the more anterior injections of IBO. The NBM lesions also extended dorsally into the ventral globus pallidus. Most of the cells in the medial septum and dorsal area of the vertical limb of the diagonal band were destroyed by the IBO injections. Similar lesions have been described previously [22,52].

DISCUSSION

BW813U produced rapid, substantial and enduring decreases in ChAT activity throughout the brain. Just 25 mg/kg produced decreases of 50% to 70% in ChAT activity within an hour. These dramatic decreases persisted for at least 5 days following a 50 mg/kg injection, and had still not recovered to normal levels by 19 days later.

ChAT activity was decreased to a greater degree by the combined use of IBO microinjections and BW813U than by either agent alone, and to a greater degree than has been previously reported in similar studies designed to investigate the relationship of the cholinergic system and memory. However, in spite of the dramatic decrease in regional levels of ChAT activity, and in contrast to studies using lesioned rats [22–24, 27, 38], choice accuracy in this spatial memory task was not impaired.

The lack of effect on behavior may be due to 2 possible factors: The radial maze retention paradigm chosen may not be sufficiently difficult or the decrease in acetylcholine production was not sufficient to affect behavior.

Although performance in the radial maze is sensitive to basal forebrain lesions and anticholinergic drugs, rats that are overtrained can tolerate higher doses of anti-cholinergic agents without showing a performance deficit [9]. Performance of a well-learned task may not require the same degree of central processing at acetylcholine synapses as does acquisition [9]. Indeed, antagonism of acetylcholine neurotransmission impaired acquisition more than retrieval of spatial memory [10]. Overtraining may make spatial memory processing relatively resistant to decreased acetylcholine production. Therefore, treatment with BW813U might have had a greater effect on acquisition in the radial maze or if delays were introduced between choices to make the task more difficult.

Although ChAT activity throughout the brain was very low, acetylcholine production might not have been decreased to the same degree. The rate of acetylcholine release is considerably less than the activity of ChAT measured *in vitro* on brain homogenates under optimal conditions [21, 49, 55]. Therefore, ChAT activity exists in greater surplus than is necessary to maintain normal acetylcholinergic neuronal activity [50]. The treatments used in the present study (i.e., BW813U and pentylenetetrazol) might not have reduced acetylcholine production and levels sufficiently to impair learning and memory. Inasmuch as acetylcholine levels were not assayed, the absolute effectiveness of these treatments cannot be determined. However, in another study, BW813U decreased whole brain acetylcholine levels by 50% and hippocampal levels by 39% when measured 1 day after a single 100 mg/kg (IP) injection (H. White, personal communication). However, the possibility remains that although whole brain acetylcholine levels were decreased, synaptic levels may be sufficient to accommodate normal neurotransmission.

Destruction of cholinergic cells in the basal forebrain by IBO injections decreased cortical and hippocampal ChAT activity to the same degree as treatment with BW813U. However, only basal forebrain lesions are associated with impaired learning and memory [1, 17, 22, 23, 27, 31, 38]. These lesions completely prevent acetylcholine production in specific regions of cortex and hippocampus. However, IBO lesions do not destroy all cholinergic cells in the NBM and MSA [52]. In contrast, the inhibition of ChAT activity by BW813U decreased acetylcholine production at all cholinergic terminals throughout the brain. Therefore, the essential difference between these two preparations was the degree of ChAT loss at a particular cholinergic terminal and the number of terminals affected. The lack of behavioral effect following BW813U treatment may be because all transmitter functions in a critical population of cholinergic terminals were not completely blocked.

The present study demonstrated that a dramatic decrease in brain ChAT activity does not impair choice accuracy in

the radial maze. Therefore, the correlation in previous experiments [1, 17, 22, 23, 27, 38, 43] between a decrease in ChAT activity and an impairment in learning and memory might not be due solely to changes in ChAT activity, but may be related to many other factors as well. For example, BW813U injections would only impair acetylcholine production and not alter production and release of other neurotransmitters that might coexist with acetylcholine, e.g., somatostatin [14]. In contrast, IBO lesions would destroy all neurotransmitter substances within the basal forebrain cells as well as nearby non-cholinergic cells that may also play a role in learning and memory. The loss of these other transmitters, acting as neuromodulators or trophic factors, may have a greater effect on information processing in the cortex and hippocampus than does the loss of acetylcholine production alone. If correct, this may explain why lesions of the NBM and MSA produced by IBO injections have a greater effect on behavior than an injection of BW813U.

ACKNOWLEDGEMENTS

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