Journal of Traditional Chinese Medicine 2(4): 277-284, 1982.

EFFECTS OF ANISODINE ON MEMORY

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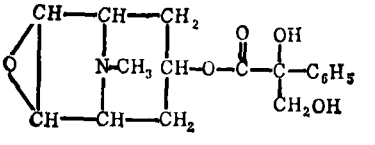
Anisodine (AT_3) is a new alkaloid isolated from Scopolia tangutica, Anisodus tanguticus, a plant of Solanaceae family, collected from Mt. Tanggute. AT_3 has anticholinergic property. The influences of AT_3 on memory behavior, hippocampal electrical activity and acetylcholine content in animals were studied. The evaluation of memory deficits was based on the following: (1) percentage of conditioned passive avoidance reaction (CAR), (2) mean response time, (3) mean ability to perform correctly 5 successive times in 10 trials, (4) mean number of trials to correctly perform 9 out of 10 trials. It was found that AT_3 influences unconsolidated memory (three day training period) more than consolided memory (six day training period). It was also observed that the mnemonia effects of AT_3 was associated with ECoG changes and cholinergic reduction in hippocampus. The major findings arising from studies of hippocampal electrical activity in orienting experiments is that hippocampal electrical response decreased after AT_3 injection. These findings suggested that the chronic administration of AT_3 raises the possibility of impairing the ability of patients, particularly elderly ones, to learn new material and to store, or acquire new information into long-term memory.

Anisodine (AT_3) is a new alkaloid isolated from *Scopolia tangutica, Anisodus tanguticus,* a plant of *Solanaceae* family, collected from Mt. Tanggute. The efficacy of AT_3 has been well established in treating migraine, CO poisoning, spasm of retinal blood vessels, ischemic optic neuropathy, inflammatory diseases of the nervous system, acute cerebral vascular accidents, organic phosphorus poisoning, as well as in Chinese traditional drug anesthesia. The structural formula of AT_3 demonstrates a hydroxyl group on the α-carbon atom of the tropic acid moiety.[1]



AT_3 has also anticholinergic property.[1] The cholinergic mechanisms play an important role in cognitive activities such as learning and memory.[2] It is still controversial whether the chronic administration of AT_3 may affect memory. In order to evaluate the mnemonic effects of AT_3, we studied the influences of AT_3 on memory behavior, hippocampal electrical activity and acetylcholine (ACh) content in animals. The results are presented in the following.

EFFECT OF AT_3 ON MEMORY BEHAVIOR

Materials and Methods

The same apparatus and procedures of a Ψ -maze brightness discrimination task were employed as in our previous learning and memory experiments.[3] Forty-two male rats weighing 200-300 g which performed correctly in 8 out of 10 trials after three days of learning trials were divided into two experimental groups. All test sessions for a single subject were scheduled at the same time of day.

Procedures

The first experimental group (n=19) was subdivided into drug group A (n=10) and control group A (n=9). Immediately after the training trials on the third learning day, rats of drug group A received a single dose of AT_3 (1mg/kg i.p. in 10 ml saline), while control group rats received only saline.

Then each rat was retested and given 10 trials per day at different time intervals (i.e., at 1hr, 1d, 2d, 3d, 4d, 5d, 6d, and 7d after intraperitonially injected with AT_3 or saline.)

The second experimental group (n=23) was subdivided into drug group B (n=13) and control group B (n=10). Each rat was retested by the same procedures in the first set of experiment except that the learning trials is doubled to 6 days (also 10 trials per day) before recall test.

The evaluation of memory deficits was based on the following: (1) percentage of conditioned passive avoidance reaction (CAR), (2) mean response time, (3) mean ability to perform correctly 5 successive times in 10 trials, (4) mean number of trials to correctly perform 9 out of 10 trials.

Results

Experiment 1

1. Mean response time and percentage of CAR

After an initial one hour of drug administration, the mean response time increased from 2.78 ± 0.23 sec (S.E.M.) to 3.65 ± 0.18 sec (S.E.M.), and the percentage of CAR reduced to 71.00 ± 5.54% (S.E.M.). The mean response times and percentages of CAR at different time intervals after drug injection showed very significant differences ($F=6.561$, $P<0.01$; $F=8.423$, $P<0.01$ respectively). Q-test showed that the mean response time was the longest, 4.43±0.31 sec (S.E.M.), and the percentage of CAR was the lowest, 58.00 ± 4.42% (S.E.M.), on the first retest day after drug administration, whereas in control animals increased amount of training at the retest produced improvement in scores. Data obtained at 1hr, 1d, and 2d after drug administration revealed that drug-treated rats were significantly recall-impaired as compared with the control animals. All other possible pairs of comparisons proved to be insignificant, Tables 1 and 2, and Figures 1 and 2 show these results.

2. Mean number of trials to correctly perform 9 out of 10 trials

The drug-treated animals required 40 training trials on the average to reach the

Table 1. Effect of anisodine on mean response time

Time after injection	Anisodine A	Control A	t	P
1 h	3.65±0.18	2.83±0.33	2.225	< 0.05
1 d	4.43±0.31	2.89±0.33	3.777	< 0.01
2 d	4.14±0.22	3.11±0.10	4.082	< 0.001
3 d	3.28±0.20	3.23±0.19	0.168	> 0.05
4 d	3.29±0.18	2.95±0.29	1.028	> 0.05
5 d	3.09±0.13	2.95±0.29	0.477	> 0.05
6 d	3.07±0.14	2.98±0.17	0.455	> 0.05
7 d	3.27±0.16	3.13±0.29	0.417	> 0.05

The figures denote mean±S.E.M. (sec).

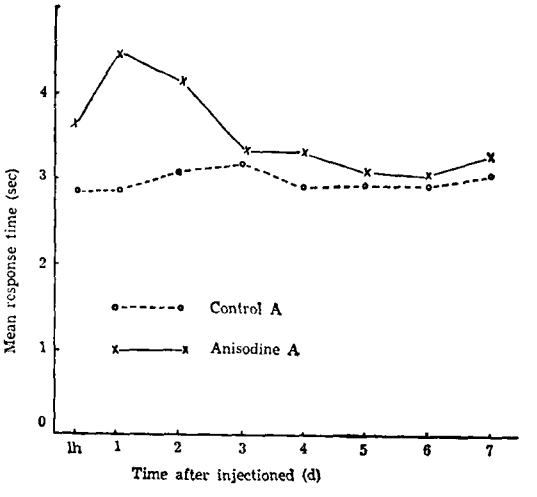


Fig. 1 Effect of anisodine on mean response time

criterion; the control animals, 13 trials. The difference between the two groups was highly significant ($t=3.536$, $P<0.01$). (Fig. 3)

3. Mean ability to succeed 5 successive times in 10 trials

The ability to perform consecutive errorless trials is an important parameter to evaluate memory. Here AT_3 had a pronounced debilitating effect. On the first retest day only two rats in the drug group reached this criterion, opposed to 7 in the control group.

Table 2. Effect of anisodine on percentage of CAR

Time after injection	Anisodine A (%±S.E.M.)	Control A (%±S.E.M.)	t	P
1 h	71.00±5.40	87.78±3.24	2.729	< 0.01
1 d	58.00±4.42	85.56±1.76	5.557	< 0.001
2 d	63.00±8.03	85.56±2.24	2.564	< 0.05
3 d	83.00±3.67	84.44±2.42	0.320	> 0.05
4 d	88.00±2.49	88.89±3.51	0.210	> 0.05
5 d	90.00±2.11	91.11±2.61	0.334	> 0.05
6 d	86.00±1.67	91.11±2.00	1.973	> 0.05
7 d	87.00±3.00	86.67±2.36	0.085	> 0.05

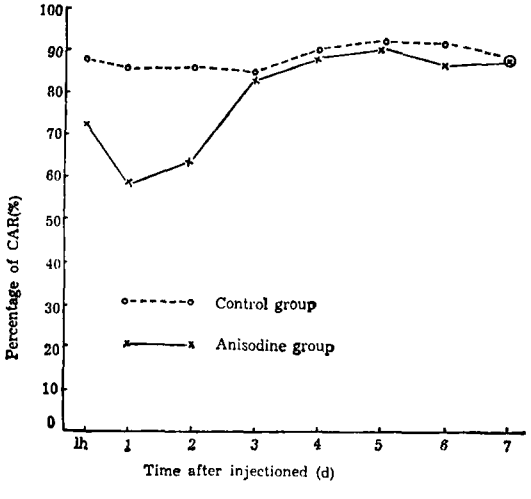


Fig. 2 Effect of anisodine on percentage of CAR

On the second retest day in drug group, only three rats reached the criterion, opposed to 9 in the control group (according to exact method, $P=0.0185$ and $P=0.0024$ respectively) (Table 3).

Experiment 2

1. Mean response time and percentage of CAR

Although a trend toward increased mean response time and decreased percentage of CAR were found in drug group B, the experiment results of drug group B and control

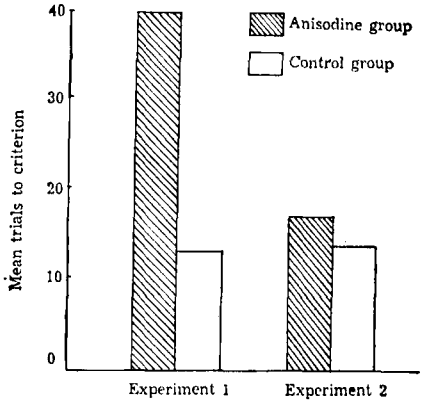


Fig. 3 Effect of anisodine on mean trials to 9 out of 10 trials correct criterion

Table 3. Effect of anisodine on the ability to reach 5 out of 10 trials consecutive correct criterion

Time after injection	Anisodine A (N = 10)	Control A (N = 9)
1 h	7	8
1 d	2*	7
2 d	3**	9
3 d	7	9
4 d	10	9
5 d	10	8
6 d	10	9
7 d	9	9

The figures denote number of animals to achieve criterion of consecutive correct responses.

* $P = 0.0185$

** $P = 0.0024$

group B were not significantly different except for the first retest day (Tables 4 and 5).

2. Mean number of trials to correctly perform 9 out of 10 trials

Drug group B required 17 training trials on the average to achieve this criterion; 14 for control group B (Fig 3). There was no significant difference between these two groups (t=1.901, P>0.05).

Table 4. Effect of anisodine on mean response time

Time after injection	Anisodine B	Control B	t	P
1 h	2.99±0.34	3.01±0.34	0.039	> 0.05
1 d	3.48±0.36	2.34±0.20	2.533	< 0.05
2 d	3.52±0.32	2.93±0.27	1.352	> 0.05
3 d	3.82±0.31	3.16±0.21	1.658	> 0.05
4 d	3.60±0.26	3.14±0.18	1.377	> .005
5 d	3.76±0.38	3.05±0.27	1.427	> 0.05
6 d	3.78±0.27	3.43±0.29	0.885	> 0.05
7 d	3.48±0.33	3.37±0.26	0.256	> 0.05

The figures denote mean±S.E.M. (sec).

Table 5. Effect of anisodine on percentage of CAR

Time after injection	Anisodine B (%±S.E.M.)	Control B (%±S.E.M.)	t	P
1 h	85.38±4.41	88.00±4.16	0.417	> 0.05
1 d	73.85±7.47	95.00±2.24	2.409	< 0.05
2 d	75.38±6.16	88.00±2.00	1.734	> 0.05
3 d	72.39±6.81	85.00±3.42	1.520	> 0.05
4 d	75.38±5.26	88.00±3.27	1.892	> 0.05
5 d	70.00±8.84	84.00±3.40	1.326	> 0.05
6 d	74.62±5.73	82.00±4.90	0.943	> 0.05
7 d	78.46±4.51	88.00±3.59	1.580	> 0.05

Table 6. Effect of anisodine on the ability to succeed 5 successive times in 10 trials

Time after injection	Anisodine B (N = 13)	Control B (N = 10)
1 h	10	9
1 d	7*	10
2 d	10	10
3 d	10	10
4 d	11	9
5 d	10	9
6 d	11	8
7 d	11	10

The figures denote number of animals that achieve criterion of 5 consecutive correct responses.

* P = 0.0167

3. Mean ability to succeed 5 successive times in 10 trials

No significant differences were observed on this measure except for the first retest day (exact method, P=0.0167). These data are shown in Table 6.

EFFECT OF AT_3 ON HIPPOCAMPAL ELECTRICAL ACTIVITY

Materials and Methods

Twelve adult male rabbits weighing 2-3 kg were divided into two groups of 6 each. The experimental group received AT_3 0.25mg/kg in 0.25 ml saline i.v.; the control group received the same volume of saline only. Five to six days prior to the experiment all animals were implanted bilaterally with 4 pairs of electrodes, two pairs in the dorsal hippocampus, one in the sensory area and another in occipital region. The rabbits were gently and frequently handled during a 5-6 day recovery period.

Procedures

To assess the dorsal hippocampal and neocortical electrical responses to novel stimuli, 6 drug-treated animals were given 20 acoustic stimuli (a 75 dB, 250 Hz pure tone from an audiogenerator, 4 sec) one hour after intravenously administrated AT_3 and 20 touch stimuli (250 g, 4 sec) 24 hours later, at

intervals of 30-60 sec. Six control animals had received intravenous injection of saline before novel stimuli given.

Results

An abbreviated presentation of the data is given below.

In the orienting experiment, the dorsal hippocampal tracings reflected the electrical responses to pure tones and touches as novel stimuli by shifting to a regularized rhythmic θ wave activity at a frequency of 5-6 Hz, from irregular high-voltage mixed slow and fast activity, whereas neocortical tracings tended to show low-voltage, relatively desynchronized. The mean percentage of electrical response to pure tones in drug-treated animals was 40±6.46, and 74.17±4.36 in controls, significant at $P<0.01$, while response to touches for drug-treated animals was 45±2.58, and 83.33±3.10 for controls, with significant difference at $P<0.001$.

EFFECT OF AT_3 ON HIPPOCAMPAL ACh CONTENT

Materials and Methods

Forty male rats weighing 200-300 g were divided into two groups. The experimental group (n=30) received AT_3 1 mg/kg i.p. in 10 ml saline, and the control group (n=10), saline only. The experimental group was subdivided into three groups (C, D and E) of 10 rats each. One hour after AT_3 administration, rats of group C were sacrified by decapitation, the brains were quickly removed and placed on an ice-cold plate, and from each brain 3 samples were isolated. These included hippocampus, caudate nucleus and cerebral cortex. The samples were weighed on a torsion balance and homogenized in a tube with a chilled mixture of eserine in Locke's solution. The bioassay of ACh was performed using leech micromethod as previously described.[4]

Forty-eight and 72 hours after drug administration, animals of group D and E were decapitated and the effect of AT_3 on ACh levels of hippocampus, caudate nucleus and cerebral cortex was studied by the same procedures. Control animals were decapitated one hours after saline injection and studied in the same way.

Results

One hour after AT_3 administration the brain ACh content of drug group C decreased and there were significant differences in the hippocampus and cerebral cortex as compared with those of controle. Forty-eight hours after AT_3 injection the hippocampal and cerebral cortical ACh contents of drug group D were higher than those of group C after one hour, but still lower than those in controls and the difference between the hippocampal ACh content of drug group D and control group was significant, while no significant difference was found between neocortical ACh content of drug group D and control group (Tables 7 and 8). Seventy-two hours after AT_3 injection there were no significant differences between drug group E and control group (Table 9).

Table 7. Effect of anisodine on ACh content in some areas of cerebrum

Cerebral area	Anisodine C (1h after injection)	Control group	t	P
Hippocampus	5.56±0.63	11.95±0.94	3.919	< 0.001
Cerebral cortex	3.96±0.30	10.41±0.63	4.925	< 0.001
Caudate nucleus	2.61±0.34	3.56±0.44	0.293	> 0.05

The figures denote mean±S.E.M. (μg/g wet brain).

Table 8. Effect of anisodine on ACh content in some areas of cerebrum

Cerebral area	Anisodine D (48 h after injection)	Control group	t	P
Hippocampus	9.24±0.69	11.95±0.94	2.679	< 0.05
Cerebral cortex	9.13±0.81	10.41±0.63	1.049	> 0.05
Caudate nucleus	2.48±0.28	3.56±0.44	2.054	> 0.05

The figures denote mean±S.E.M. (μg/g wet brain).

Table 9. Effect of anisodine on ACh content in some areas of cerebrum

Cerebral area	Anisodine E (72 h after injection)	Control group	t	P
Hippocampus	12.24±0.77	11.95±0.94	0.268	> 0.05
Cerebral cortex	10.98±1.00	10.41±0.63	0.479	> 0.05
Caudate nucleus	4.80±0.59	3.56±0.44	1.672	> 0.05

The figures denote mean±S.E.M. (μg/g wet brain).

DISCUSSION

The above memory behavior studies demonstrate that AT_3 influences unconsolidated memory (three day training period) more than consolidated memory (six day training period). In retesting, control groups A and B showed progressive improvement with successive trials. However, there was no evidence of improvement of performance during repetitions of the test in drug groups A and B; on the contrary, under different degrees of training in the brightness discrimination task, drug group rats all showed significant impairment of memory behavior. We also found a greater degree of impairment of memory in drug group A (with a low degree of training) than B (with a high degree of training).

Drug group A had a significant increase in mean response time, reduction in percentage of CAR and decreased ability in performing correctly in 5 consecutive trials out of 10 at 1h, 24h and 48h after AT_3 administration, and an impairment in the ability of succeeding in 9 out of 10 trials. In drug group B, AT_3 appeared to affect the mean response time, percentage of CAR and ability of succeeding in 5 out of 10 trials to a lesser extent, and only at 24h after AT_3 injection. There was no impairment in the ability of succeeding in 9 out of 10 trials. Although AT_3 had some peripheral anticholinergic side effects, as well documented elsewhere,[5] it is highly unlikely that these effects were responsible for the discrepancy in the degree of impairment between the two drug groups, since they received the same dose of AT_3. Therefore, it is suggested that AT_3 prevented memory consolidation during learning.

The major finding arising from studies of hippocampal electrical activity in orienting experiments is that hippocampal electrical response decreased after AT_3 injection. As an explanation of this phenomenon, it is suggested that θ rhythm and frequency shifts of θ rhythm are related to information processing and learning consolidation.[6] Bennet et al. have reported evidence which suggests that attentional sets and orienting responses related to discriminative stimuli are accompanied by hippocampal θ rhythm.[7] Brown related hippocampal activity to levels of arousal, emphasizing that θ rhythm may accompany orienting and searching behavior in a new environment.[6] Our previous studies showed that the ability to retain the passive avoidance response reduced and the rate of conditional reflex formation decreased in bilaterally cautery hippocampectomized rats, whereas no evidence of significant effect was observed between groups of rats with bilateral destruction of neocortex and control subjects.[8] Our previous studies also demonstrated that

the bilateral electrical stimulation of hippocampus interfered with the initial stage of conditional reflex formation, while bilateral electrical stimulation of neocortex revealed no influence on it.[9] We also found that the bilateral electrical stimulation of hippocampus did not impair a well-established conditional reflex.[9] These, together with the results from studies of both memory behavior and hippocampal electrical activity reported here, indicate that the patterns of learning and memory behavior produced by AT_3 may be compared to the patterns demonstrated in memory disorders produced by bilateral hippocampus stimulation or destruction, and obviously support the assumption that hippocampal function may be correlated with memory and hippocampal regions are an important substrate for memory storage.[10]

Pharmacological studies demonstrated that AT_3 is similar to scopolamine. They are powerful and specific anticholinergic agents capable of crossing the blood-brain barrier. The distribution patterns of these two drugs over various regions of the brain were also similar. The highest level was found in the hippocampus, corpus striatum and cerebral cortex.[1,5] It is very interesting that although a considerable number of animal studies have suggested that cholinergic agents may influence memory, very few investigations have attempted to correlate the neurochemical, electrophysiological and memory behavior effects of cholinergic agents.[2] The present investigations suggested that the mnemonic effect of AT_3 was associated with ECoG changes and brain ACh content reduction in animals. One hour after AT_3 injection the effect of the drug on learning and memory began, together with concomitant ECoG changes and significantly decreased ACh contents of the hippocampus and neocortex; 48 hours after injection the memory deficits persisted and the hippocampus had significantly less ACh content in rats with AT_3 injection than in controls, while the ACh content of neocortex showed no significant difference between drug and control group; and simultaneously, in orienting experiments electrical response decreased, and only hippocampal tracings showed θ rhythm and frequency shifts of θ rhythm, whereas neocortical tracings tended to be desynchronized at 1h and 24h after AT_3 injection. These findings support the view that the cholinergic activity within hippocampus is essential for the storage of new information.

Our neurochemical, electrophysiological and maze studies with AT_3 all implicate the cholinergic activity in memory and indicate (1) that cholinergic synapses represent an essential component or link in mnemonic substrate; (2) that variations in memory strength are associated with variations in cholinergic sensitivity. Similarly we infer that such states as forgetting must be characterized by reduced cholinergic excitability, since, here, AT_3 reduced the expression of memory; therefore we would predict that they should also decrease the memory strength for as long as they are active in the nervous system. This, too, is borne out by our experimental data. If the modification of memory synapses is graded rather than all-or-none, then low degree of learning should produce synapses that conduct relatively poorly, whereas increased amount of training should produce synapses that conduct well. Consequently a poorly learned habit should be blocked by a dose of anticholinergic agent that does not block the well-established habit or blocks in a low degree. That this is the case has been shown in our two behavior

data. Tables 1-6, Figures 1-3; (3) that the cholinergic reduction in hippocampus is particularly relevent to the learning and memory abnormalities of animals with AT_3, and the effect of AT_3 seems particularly localized to the storage, or acquisition, of new information into the long-term memory. Finally, the findings of this study provide some insight into possible mechanisms by which the learning and memory impairment may be produced by the administration of AT_3.

With increasing age there is diminished choline acetyltransferase (CAT). A reduction of up to 66% was found in CAT as the subject's age approached 50 years.[11] CAT is believed to be a good marker for cholinergic neurons.[11,12] Thus, deminished CAT activity, which suggests depletion of cholinergic neurons, occurs in normal aging. Therefore, it may be worth mentioning that chronic administration of AT_3 raises the possibility of impairing the ability of patients particularly elderly ones, to learn new material and to store information in the long-term memory.

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