Pharmacology Biochemistry and Behavior, Vol. 47, pp. 219–225, 1994
Printed in the U.S.A.

0091-3057/94 $6.00 + .00

Amelioration by Aniracetam of Abnormalities as Revealed in Choice Reaction Performance and Shuttle Behavior

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Received 17 August 1992

HIMORI, N. AND K. MISHIMA. *Amelioration by aniracetam of abnormalities as revealed in choice reaction performance and shuttle behavior.* PHARMACOL BIOCHEM BEHAV 47(2) 219–225, 1994.—To delineate the possible effects of aniracetam PO on abnormal behaviors, we analyzed disrupted shuttle behavior and choice reaction (CR) performance in both aged and juvenile animals subjected to an ischemic (permanent occlusion of both carotid arteries)–hypoxic (17-min exposure to 93% N_2 and 7% O_2 mixture gas) or ischemic (20-min occlusion of both carotid arteries) insult and/or treated with methamphetamine given IP. Aniracetam at single PO doses of 10 and 30 mg/kg significantly decreased the number of incorrect lever pressings induced by IP methamphetamine in young adult rats subjected to the CR test battery. A 21-day PO regimen with aniracetam (30 mg/kg/day) resulted in an increase in the number of correct responses and a decrease in the CR latency as detected in the CR task with young adult rats inflicted with an ischemic–hypoxic insult. Aniracetam (1–100 mg/kg PO) was also evaluated in the electrostimulation-induced hyperreactivity assay (an increase in the number of shuttle responses) in both juvenile and aged mice subjected to a 20-min ischemic insult; there again a significant improvement of performance was clearly observed. The outcomes of these behavioral pharmacological analyses suggest that aniracetam has the ability to normalize the disrupted behavior, cognition, and self-regulation or decision-making process in a comprehensive way.

Aniracetam Methamphetamine Choice reaction performance Shuttle behavior Ischemia Hypoxia Rat Mouse

THAT aniracetam protects the central nervous system (CNS) against a variety of insults is known and has been confirmed with a battery of diverse cognitive tests (2,4,5,12,13,15,16). Foltyn et al. (3) have reported that in clinical trials geriatric patients were resocialized and revitalized following aniracetam treatment. To date, however, we know that very few preclinical investigations on aniracetam have focused on the improvement of behavioral abnormalities, except for those related to performance deficit, learning, and memory. It is interesting, therefore, to refer to clinical works performed by Mindus et al. (17) and Kretschmar and Kretschmar (11), where the structurally similar, prototypic nootropic piracetam improved the perceptual-motor performance and psycho-organic symptoms, suggesting that piracetam has a weak antipsychotic effect.

Although the etiological aspects are still not entirely certain, intellectual impairment, emotional disturbance, and problematic behaviors such as nocturnal wandering and delirium are seen as sequelae of cerebrovascular disorders and of their accompanying symptoms. Two double-blind studies were recently reported on the treatment of the sequelae of cerebral infarction and hemorrhage with aniracetam (22,23). Those results statistically proved the benefit of aniracetam, especially for treating such psychiatric symptoms as nocturnal wandering and delirium, or emotional disturbance. The treatment of behavioral disturbances accompanying cerebrovascular disorders may frequently improve the quality of life for both patients and caregivers. In view of the above, it was of interest to investigate for the first time the ameliorative effects of a novel pyrrolidinone derivative, aniracetam, in juvenile or aged animals subjected to an ischemic–hypoxic or ischemic insult, or treated with methamphetamine, to see how aniracetam is systematically involved in the amelioration process.

A preliminary report of this work has appeared in abstract form (18).

METHOD

Animals

Animal care and use procedures were based on the regulations dictated by the Animal Care and Use Committee at Nippon Roche Research Center.

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Male ddY mice aged 8–13 weeks or about 18 months, and male Wistar rats aged 8–13 weeks were obtained from the Shizuoka Experimental Animal Laboratory and Crea Japan, and housed in groups of 10 (mice) and 3 (rats). They were used for experimentation after acclimatization for at least one week in cages in a room with a controlled temperature of 22 ± 2°C and relative humidity of 64 ± 10% with lights on from 0700 to 1900. The animals were given free access to water and food; the rats chosen for the choice reaction (CR) test paradigm were placed under a restricted food intake (10–12 g/day) (CE-2, Crea Japan).

According to the procedure reported by Lorez et al. (14), cerebral ischemia was caused in trained rats (choice reaction performance) by permanent occlusion of both carotid arteries under halothane anesthesia and 24 h later by a hypoxic insult, for which they were placed for 17 min in a Plexiglas box filled with 93% N_2 and 7% O_2 mixture gas.

Transient two-vessel occlusion to cause cerebral ischemia was performed according to the procedure originally developed by Himori et al. (9). During anesthesia with hexobarbital sodium (120 mg/kg IP) an occluder and releasers were loosely placed around both common carotid arteries. Four or five days later the occluder was tightened to occlude the arteries for 20 min and then loosened again by pulling the releasers to allow for reperfusion while mice were conscious.

Choice Reaction Performance in Rats

The CR performance-analysing apparatus was composed of 14 Skinner boxes placed in dark, soundproof compartments. Each Skinner box was equipped with two response bars (pressing levers), cue lamps, and a pellet dispenser. The pellet dispenser released a 45-mg pellet only when the fasting test animals pressed the correct lever.

Data were processed via an interface-controller system (a slave controller) consisting of a central processing unit, ROM (read-only memory), and RAM (random access memory). These processed data were collected and analyzed with a personal computer (PC-9801, NEC, Tokyo, Japan) using a system program written in BASIC. The commands and parameters for the experimental schedule were transferred from the master controller (personal computer) to the slave controller through a communication controller. The software for the real-time experiment control executed by the slave controller was designed and composed for this assay system in collaboration with Muromachi Kikai (Tokyo). Wide-angle charged-couple-device (CCD) cameras were installed in two Skinner boxes enabling us to observe behavioral changes in the animals during the training session. In this system for one to two months prior to the actual study all the rats were first trained to press either of the two levers by varying the "correct" lever; a cue lamp was randomly lighted above the "correct" lever with a continuous reinforcement schedule of a fixed ratio 1. The animals were considered trained when they had gained the ability to produce 80% correct responses and fewer than 15 total lever pressings during the combined intertrial interval (ITI) and differential reinforcement of other behavior (DRO) periods, and had a CR latency between 1.3 and 1.8 s in three successive sessions in the procedure, as described below. Then those highly trained animals had their CR performance (accuracy and speed) to an acquired task (pressing a lever signaled by a cue lamp) tested as shown in Fig. 1.

The CR task was begun with a DRO period (random, 2–5 s) during which the animals had to refrain from pressing either of the two levers. If they repeatedly pressed the levers

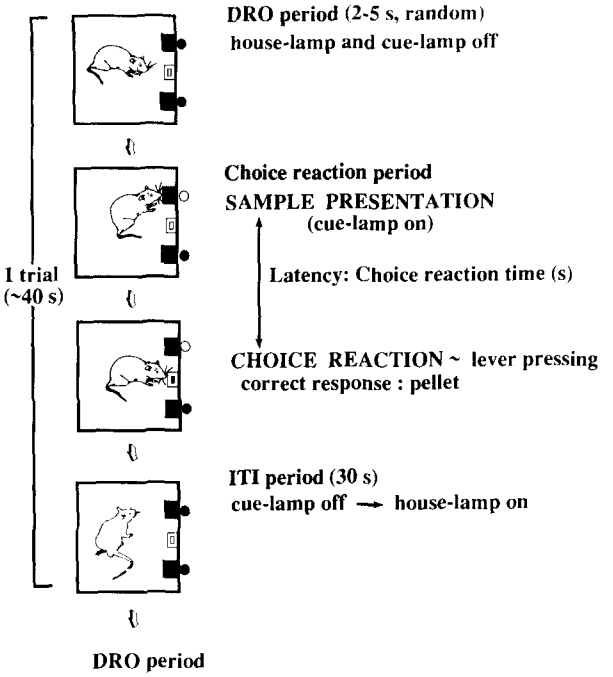

FIG. 1. The procedure of choice reaction time measurement in a behavioral study. A trial consists of three periods: differential reinforcement of other behavior (DRO), choice reaction, and intertrial interval (ITI). One trial lasts about 40 s, and a daily session comprises about 30 trials each with an ITI of 30 s. Each box represents a Skinner box showing the various stimulation configurations during each phase of a trial. Two levers are drawn as black rectangles located at the front wall of the Skinner box. A darkened box shows an off house lamp. Circles next to the levers represent cue lamps, either on (open) or off (closed).

(>10 s) during the DRO period, that trial was terminated and was followed by the ITI period. During the CR period (maximum, 10 s), the time between sample presentation with the cue lamp on and the correct lever pressing was defined as CR latency, and a food pellet (reinforcement) was provided through the pellet dispenser. With further lever-pressing responses, a house lamp was illuminated and the ITI period (30 s) was begun. One trial took approximately 40 s, and each test session consisted of 30 trials. The variables measured were the numbers of correct (CR period) and incorrect lever pressings (DRO and ITI periods) and the CR latency (in seconds) during the CR measurement period.

Thirty minutes after IP treatment with methamphetamine, the trained animals were placed in Skinner boxes and given a 5-min adaptation period. Then the CR test paradigm was begun as shown in Fig. 1. Aniracetam (1–30 mg/kg) or vehicle was given PO to the animals 30 min before the IP methamphetamine. Control values were obtained from the same animals given the vehicle only twice, one day before the drug sessions under the same test schedule.

In the tests with ischemic-hypoxic rats, aniracetam (30 mg/kg/day for 21 days) or vehicle was given PO 1 h before a 5-min adaptation period in the Skinner box, which was followed by the test session consisting of 30 trials. The CR performance was analysed every day for 21 days. The data were compared with those obtained from the vehicle-treated ischemic-hypoxic rats (control).

Shuttle Behavior in Mice

The shuttle behavior measurement apparatus was also computer-controlled and was composed of three shuttle boxes (placed in soundproof compartments) connected to a personal computer (PC-9801, NEC) via a SUB-CPU interface. Each shuttle box was equipped with a compartmentalized flip-flop electrifiable grid floor. Thus, the shuttle behavior of test animals receiving avoidable electroshocks (0.2 mA for 1 s) at 1 min intervals for 5 min was quantitatively measured for a period of 15 min by counting the number of times the floor moved over the fulcrum in the shuttle box. The electroshocks were delivered by using a shock generator/scrambler (SGS-002, Muromachi Kikai) connected to the interface. The shuttle behavior of the juvenile or aged mice, with or without ischemic insults, was measured 16–24 h after the 20-min ischemia.

Aniracetam (1–100 mg/kg) or vehicle was administered PO to animals 1 h before testing and then the animals were placed in shuttle boxes to observe their shuttle behavior for 15 min immediately after the delivery of electroshocks.

Drugs

The drugs used were aniracetam (Ro 13-5057, 1-*p*-anisoyl-2-pyrrolidinone (F. Hoffmann-La Roche, Basel, Switzerland) and methamphetamine (*d*-methamphetamine sulfate, Dainippon, Osaka, Japan). Aniracetam was suspended in 0.25% carboxymethylcellulose (CMC) solution, and methamphetamine was dissolved in 0.9% physiological saline solution.

Data Analysis

Results are expressed throughout the text as either the absolute or percent incidence of behavioral changes or means ± SEs. The following statistical analyses were made to assess the differences in values between groups: Duncan's *t* test and two-way analysis of variance (ANOVA) followed by a Student's *t* test for CR performance analysis when overall significance was observed in the ANOVA, and multiple analysis by Dunnett, Fisher's exact probability test, and Student's *t* test for shuttle behavior analysis. A *p* value less than 0.05 was considered to be statistically significant.

RESULTS

Incorrect Lever Pressing in Choice Reaction Performance of Rats Treated With IP Methamphetamine

An IP injection of methamphetamine at doses of 1 and 3 mg/kg produced abnormal behaviors in CR performance in terms of a decreased number of correct responses, an increase in the number of lever pressings during the ITI and DRO periods (incorrect lever pressing), and a lengthening of CR latency (Fig. 2). Based on gross behavior and reproducibility of the observed behavioral changes, we regarded the increase in the number of incorrect lever pressings caused by 1 mg/kg methamphetamine as an appropriate variable in the test procedure to evaluate the efficacy of aniracetam. As clearly seen in Fig. 3, aniracetam (1, 10, and 30 mg/kg PO) improved the behavioral disorders caused by methamphetamine (1 mg/kg IP) in a dose-dependent manner, $F(3, 42) = 8.086$, $p < 0.01$, which was statistically significant ($p < 0.01$) at higher doses. Aniracetam per se neither decreased nor increased the number of lever pressings during the session.

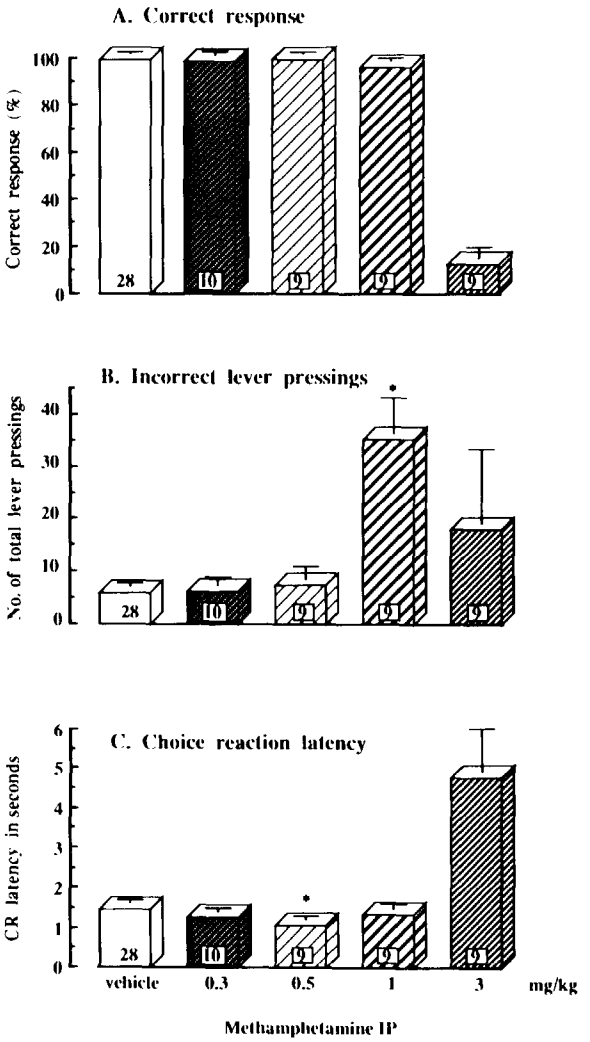

FIG. 2. Effects of methamphetamine on the choice reaction task in rats. Methamphetamine was injected IP 30 min prior to the test. In each test session, the correct response (A), the number of total incorrect lever pressings (B), and mean choice reaction latency of correct responses (C) were measured. Each value represents the mean ± SE. The animal number in each group is shown in each column. *$p < 0.01$ vs. their corresponding vehicle-treated groups (Duncan's *t* test).

Correct Response and Reaction Time in Choice Reaction Task With Ischemic-Hypoxic Rats

Among the 56 trained rats, 9 were allocated to the sham-operated group and the rest were inflicted with an ischemic-hypoxic insult. Thirteen of the 47 animals died within seven days, reaching approximately 30% mortality. In the computerized tasks, both speed and accuracy in their CR performance were impaired. Among the surviving 34 rats, 19 animals giving fewer than 60% correct responses were regarded as functionally disrupted and were subjected to the efficacy evaluation study on aniracetam (a 21-day regimen at a daily PO dose of 30 mg/kg). Long-term treatment with aniracetam resulted in an increase in the number of correct responses; the percent difference between the vehicle- and aniracetam-treated groups became significant as the period of treatment lengthened, $F(1, 119) = 22.645$, $p < 0.01$ (Fig. 4); 36.2 ± 13.6%

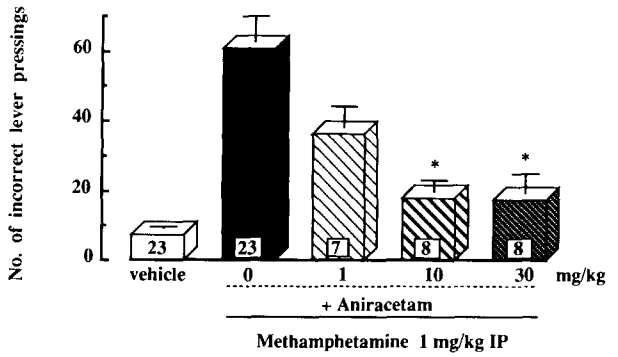

FIG. 3. Effects of a single PO administration of aniracetam on the number of total incorrect lever pressings induced by methamphetamine (1 mg/kg IP) in rats. Methamphetamine was injected IP 30 min prior to the test and aniracetam (1, 10, and 30 mg/kg PO) 1 h prior. In this experiment for the efficacy evaluation of aniracetam, high responders to methamphetamine were chosen, and thus the first and second studies for the selection and efficacy evaluation were carried out one week apart. Each value represents the mean ± SE. The animal number in each group is shown in each column. $*p < 0.01$ vs. vehicle (Duncan's t test).

for the vehicle-treated group and 61.2 ± 17.1% for the aniracetam-treated group in the final seventh block ($p < 0.01$). The delay in CR latency in rats with ischemic-hypoxic injury was also improved by aniracetam, $F(1, 119) = 6.693$, $p < 0.05$, as seen in Fig. 5. A single PO administration of aniracetam failed to cause significant changes in this test paradigm.

Electrostimulation-Induced Shuttle Response in Juvenile or Aged Mice Subjected to Cerebral Ischemia

A hyperreactive response to electrical stimulation in shuttle behavior was quantitatively determined using an active avoidance test apparatus for a period of 15 min. In mice, an excessive increase in shuttle response after the 20-min cerebral ischemic insult was observed regardless of age. The abnormal response was significantly reduced in both juvenile (9–14 weeks old) and aged mice (about 18 months old) treated with PO aniracetam (Table 1).

DISCUSSION

A great deal of attention has been given to changes in the human brain that may contribute to the age-dependent decline in brain organizational functions. One such factor is age-related impairment of cerebrovascular integrity, which becomes striking when viewed on a regional basis (21). Since brain metabolism, cerebrovascular circulation, and function are usually regarded as being tightly coupled, it is generally accepted that the progressive decline in the brain's functional competence is reflected in circulatory and metabolic changes (31). Notably, in this study aniracetam improved behavioral abnormalities observed in rats with an ischemic-hypoxic insult as well as in senescent mice with cerebral ischemia (Table 1, Figs. 4 and 5). Age-related functional impairments in senescent or hypoxic animals have been proposed as models for those in humans (6,24). A stroke-prone strain of spontaneously hypertensive rats (SP-SHRs) has been also characterized as a model for cerebrovascular disease, as more than 80% of the males spontaneously experience cerebral hemorrhages and infarcts (7). Kubota et al. (2) have also demonstrated that in both rats aged 15–20 months and SP-SHRs aged 26–27 weeks, which have longer latency times to make the correct response than do age-matched Wistar rats, aniracetam significantly shortened the choice reaction latency. From these findings, it can be concluded that aniracetam has broad spectrum activity in normalizing disrupted brain functions in animals with clinically observable functional impairments.

Our results with aniracetam might be, in part, indicative of a specific memory-improving effect of aniracetam, as observed by Perio et al. (25) in the study on the social interaction

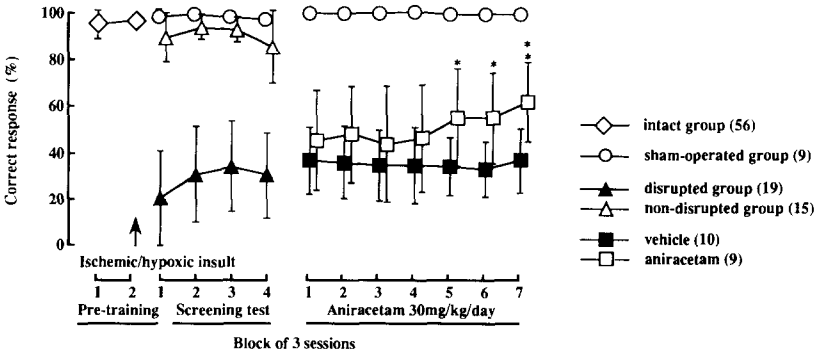

FIG. 4. Effects of chronic treatment with aniracetam on the correct response in the choice reaction task by ischemic-hypoxic rats. The screening test was started two weeks after ischemic-hypoxic insults. The treatment separated the test animals into disrupted ($n = 19$) and nondisrupted ($n = 15$) groups. For the efficacy evaluation, aniracetam at a dose of 30 mg/kg ($n = 9$) or vehicle ($n = 10$) was administered PO once a day to the disrupted group for 21 days. Test sessions (30 trials) were performed every day, with the PO administration done 1 h before the first trial. Each point represents the mean of correct responses during three sessions (one session/day). The vertical lines represent ±SEs. The animal number in each group is shown in parentheses. $*p < 0.05$, $** p < 0.01$ vs. their corresponding vehicle-treated groups (significant two-way analysis of variance followed by Student's t test).

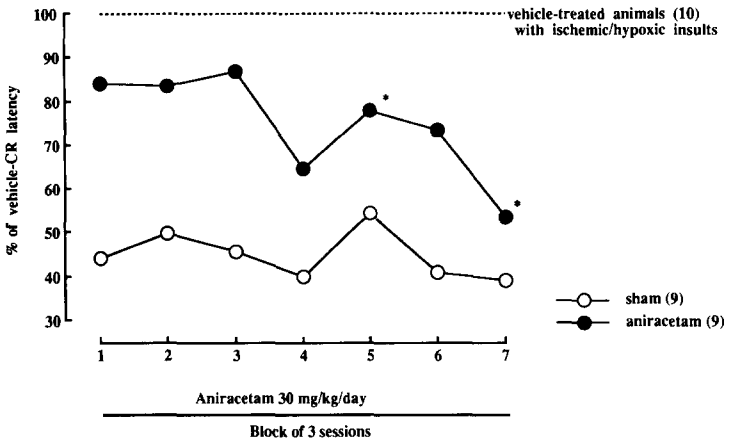

FIG. 5. Effects of chronic treatment with aniracetam on percent change in the choice reaction (CR) latency of the vehicle-treated animals with an ischemic-hypoxic insult. The screening test was started two weeks after an ischemic-hypoxic insult. Aniracetam at a dose of 30 mg/kg or vehicle was administered PO once a day to the CR performance-disrupted group for 21 days. Experiments were conducted daily, and the PO administration was done 1 h before the start of the test session consisting of 30 trials. Each point represents the mean of CR latency during three sessions (one session/day). The animal number in each group is shown in parentheses. *$p < 0.05$ vs. their corresponding vehicle-treated ischemic-hypoxic groups (significant two-way analysis of variance followed by Student's t test).

between an adult rat and a juvenile conspecific. Furthermore, aniracetam has been delineated as a cognition enhancer; it improves cognitive functions that have been impaired by different procedures and in different phases of information processing (2,4,5,12,13,15). Aniracetam may also contribute to improving consciousness, self-regulation, and the decision-making process. In fact, in a computerized EEG analysis with rats, Santucci et al. (29) clearly demonstrated that aniracetam increased mean integrated power in the 16–20 and 20–32 Hz bands, and decreased the low and middle range frequency bands, a profile indicative of vigilance-enhancing properties. This evidence is supported by the experimental results of ours

TABLE 1

SUPPRESSION OF ELECTROSTIMULATION-INDUCED HYPERREACTIVITY BY ANIRACETAM IN JUVENILE AND AGED MICE WITH ISCHEMIC INSULTS

Group/Treatment	Dose (mg/kg PO)	No. of Animals Employed	No. of Shuttle Responses	No. of Subjects/No. of Animals Employed		
				With a Value Less Than MV	With a Value Higher Than MV by 25%	With a Value Higher Than MV by 50%
Juvenile mice (9–14 weeks old)						
Sham-operated	–	23	14.2 ± 2.5*	12/23†	16/23‡	20/23‡
Ischemia + Vehicle	–	22	34.9 ± 4.2	1/22	4/22	9/22
+ Aniracetam	1	21	25.5 ± 6.5	8/21§	12/21§	13/21
	10	23	18.3 ± 3.1¶	11/23‡	16/23‡	17/23
	100	22	17.0 ± 3.0¶	11/22‡	16/22†	16/22
Aged mice (~18 months old)						
Sham-operated	–	34	9.2 ± 1.2*	19/34†	28/34†	32/34†
Ischemia + Vehicle	–	27	36.7 ± 4.6	0/27	2/27	8/27
+ Aniracetam	1	23	19.8 ± 4.4¶	9/23†	15/23†	16/23†
	10	23	29.6 ± 5.6	3/23	8/23§	14/23
	100	24	20.9 ± 4.2¶	7/24‡	11/24‡	17/24‡

Four to five days after surgical operation, transient cerebral ischemia was given to male ddY mice by occluding the common carotid arteries for 20 min. Experiments were carried out 16–24 h later. One hour after PO administration of aniracetam suspended in 0.25% CMC solution, mice were placed in a shuttle instrument supported with a computer system and received avoidable electroshocks (0.2 mA for 1 s) at intervals of 1 min for 5 min, following which shuttle response of the animals was counted for a period of 15 min. MV = the mean value of sham-operated group (14.2 ± 2.5 for juvenile mice, 9.2 ± 1.2 for aged mice). *$p < 0.01$ vs. respective ischemia control groups (Student's t test). †$p < 0.001$, ‡$p < 0.01$, and §$p < 0.05$ vs. respective ischemia control groups (Fisher's exact probability test). ¶$p < 0.05$ vs. respective ischemia control groups (multiple analysis by Dunnett).

and others that aniracetam acted to shorten the choice reaction latency, which is a measure of functional arousal (i.e., the selective perception enabling us to exclude certain features of the environment from other features). The choice reaction performance is also considered to evaluate decision-making and cognition of test subjects.

There are several alternative explanations for the effects of aniracetam. Cerebral hypoxia or ischemia might reasonably be expected to reduce energy formation essential for protein and neurotransmitter biosyntheses and ion transport, thus profoundly disrupting neurotransmitter metabolism and neuronal degeneration and thereby reducing the capacity to maintain such functions as ionic balance and neurotransmission (8,35). Therefore, preserving neuronal integrity or synapse-synapse interaction is one of the main targets for improving disrupted brain functions. A brain contains more than 10^{15} synapses, which are pivotal points for interneuronal communication. Aniracetam might be expected to enhance synapse efficiency. Incidentally, when Schaffner attempted to observe such an effect with aniracetam, he found the primary cortical response evoked by sciatic nerve stimulation in rats was modestly enhanced by IV aniracetam (unpublished observation). Long-term in vitro potentiation of synaptic transmission (mossy fiber-CA_3 synapses) in the hippocampus was augmented under the effect of aniracetam (30). These data suggest that aniracetam facilitates responses to externally applied or internally occurring stimuli through central structures.

Furthermore, aniracetam has been reported to counteract glutamate-induced neurotoxicity in rat cerebellar granule cells through its potentiating action on the metabotropic glutamate receptor-coupled stimulation of phospholipase C, as revealed by the measurement of ^{3}H-IP formation (27). This would presumably also lead to a noticeable ameliorating effect on problematic CR performance of ischemic-hypoxic rats (21-day treatment with aniracetam).

In a broad assessment of receptor binding affinities, aniracetam exhibited no appreciable binding affinity for adenosine subtypes, α- and β-adrenergic, dopamine, cholinergic muscarinic, opiate, serotonine, and glutamate subtypes, benzodiazepine, or GABA receptors, among others (16), thereby excluding the possibility that aniracetam acts directly via some of the known neurotransmitter systems. Long-term treatment with aniracetam, however, resulted in a significant decrease of ^{3}H-QNB binding in rat dentate gyrus (19). The action mechanism is still not thoroughly understood, although it has been suggested to involve indirect facilitation of cholinergic neurotransmission (as a neuromodulator), especially under conditions of dysfunction (19,33,36). Results from several lines of research have suggested that piracetam or aniracetam influences DAergic neurotransmission—that is, the potentiation of haloperidol-induced catalepsy (28) and blockage by sulpiride of a decrease in locomotor activity (1), enhanced release of prolactin (20), and reduced DA levels and delayed DA turnover (26). In this study, aniracetam was active in ameliorating the disrupted choice reaction performance induced by methamphetamine (Fig. 3). Methamphetamine has been well characterized as a CNS stimulant and/or a potent CNS toxin, depending on dose used, that affects the functions of the DAergic system through a variety of actions, such as agonist action on DA receptors, depletion of DA, decrease in the number of DA transport pumps, and decrease in tyrosine hydroxylase activity. These experimental results together with clinical findings (11,22,23,32,34) would favor the hypothesis that aniracetam, like piracetam, additionally has a modulatory action on the DAergic system. An alternative explanation might be that aniracetam decreases the number of lever pressings. However, the possibility can be ruled out because aniracetam per se did not affect the lever-pressing characteristics of the test animals. Details of this mechanism are the subject of ongoing studies.

Our complete evaluation of the safety pharmacology of aniracetam demonstrated the absence of any relevant adverse effects in an extensive series of pharmacological evaluation test paradigms extending from cardiovascular, renal, and gastrointestinal systems to CNS variables. These excellent safety characteristics make it suitable even for the treatment of geriatric patients (10,22,23).

In summary, aniracetam was found to reduce behaviors which deviate from acquired behavioral patterns in trained animals subjected to methamphetamine or an ischemic-hypoxic insult. The hyperreactive shuttle behavior was also improved under the effect of aniracetam. The amelioration by aniracetam is probably due to its broad effects on self-regulation, cognition, functional arousal, or decision-making. Several mechanisms such as enhancement of synapse efficiency, neuromodulatory actions on cholinergic, DAergic, or glutamatergic and other known neurotransmission systems, or removal of receptor desensitization could be involved, although this is still speculative.

ACKNOWLEDGEMENTS

We thank Mr. Y. Tanaka for his advice as well as for his excellent technical assistance.

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