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Aniracetam: Its Novel Therapeutic Potential in Cerebral Dysfunctional Disorders Based on Recent Pharmacological Discoveries

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ABSTRACT

Aniracetam is a pyrrolidinone-type cognition enhancer that has been clinically used in the treatment of behavioral and psychological symptoms of dementia following stroke and in Alzheimer's disease. New discoveries in the behavioral pharmacology, biochemistry and pharmacokinetics of aniracetam provided new indications for this drug in the treatment of various CNS disorders or disease states. This article reviews these new findings and describes the effects of aniracetam in various rodent models of mental function impairment or cerebral dysfunction. Also, several metabolites of aniracetam have been reported to affect learning and memory in animals. It is, therefore, conceivable that major metabolites of aniracetam contribute to its pharmacological effects. The animal models, used in pharmacological evaluation of aniracetam included models of hypoattention, hypovigilance-arousal, impulsiveness, hyperactivity, fear and anxiety, depression, impaired rapid-eye movement sleep, disturbed temporal regulation, behavioral performance, and bladder hyperactivity. These are models of clinical disorders or symptoms that may include personality disorders, anxiety, depression, posttraumatic stress disorder, attention-deficit/hyperactivity disorder, autism, negative symptoms of schizophrenia, and sleep disorders. At present, there is no convincing evidence that promising effects of aniracetam in the animal models will guarantee its clinical efficacy. It is conceivable, however, that clinical trials will demonstrate beneficial effects of aniracetam in the above listed disease states. New findings regarding the mechanism of action of aniracetam, its central target sites, and its effects on signal transduction are also discussed in this review article.

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INTRODUCTION

In general, currently available cognition enhancers or nootropics have shown a major discrepancy between their therapeutic potential and predictions based on preclinical research, especially in their efficacy in the treatment of learning and memory impairments (68,80). Aniracetam (1-*p*-anisoyl-2-pyrrolidinone) is a member of a group of cognition enhancers with a pyrrolidinone moiety; it is a derivative of piracetam. Aniracetam has been clinically used in the treatment of stroke in Japan and of Alzheimer's disease in Europe. In Japan, the drug was prescribed for eight years to treat emotional disturbances, such as depressed mood and anxiety/agitation, but not memory impairment following cerebral infarction (34). Unfortunately, aniracetam (Draganon®) has been withdrawn from the Japanese market because of the unexpected failure in the latest placebo-controlled double-blind study.

Since its pharmacology was reviewed (18,34,42), a number of behavioral and biochemical findings have been reported for aniracetam, and a cholinergic mechanism was proposed as its major mechanism of action. Accumulated evidence has also suggested that aniracetam is a dual allosteric modulator of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors *in vitro* and *in vivo* (6,14,34,39,42) and of metabotropic glutamate (mGlu) receptors *in vitro* (70,71).

Aniracetam, administered systemically, is rapidly metabolized and converted to 2-pyrrolidinone and *p*-anisic acid in rats (64) and to *N*-anisoyl-γ-aminobutyric acid (*N*-anisoyl-GABA) in humans (75). These major metabolites and the parent compound were detected in the brain and cerebrospinal fluid of rats and humans (43). The pharmacokinetic properties of aniracetam make it difficult to identify the active substances in the brain, and to clarify the mode of action and target sites of aniracetam and its metabolites.

The aim of the present review is to summarize the recent behavioral and biochemical findings with aniracetam and its metabolites in animal models of neuropsychiatric disorders, and to discuss outstanding problems including novel mechanisms of action. Based on the pharmacological results, new clinical uses are proposed.

BEHAVIORAL PHARMACOLOGY

Hypoattention/Hypovigilance

Attention deficit and low vigilance are pivotal components of various mental dysfunctions in geriatric patients as well as in those with various CNS disorders. Such symptoms may often provide a psychopathological basis for many psychiatric syndromes, such as emotional disturbance, cognition impairment, sleep disorders and behavioral abnormalities (37,79). The biochemical mechanisms underlying attention and vigilance impairments are likely to be related to abnormal neurotransmission in central cholinergic, dopaminergic or serotonergic systems (9,20,21,74,78).

To assess the attention and vigilance functions a two-lever choice reaction task, which consists of a serial operation procedure based on light-dark discrimination and lever-pressing, is used in rats (56). In this task, scopolamine, 0.3 mg/kg i.p., a muscarinic acetyl-

TABLE 1. Effects of aniracetam and its major metabolites on attention and vigilance functions in the two-lever choice reaction task performed by rats

Inducer	Compound	Effective dose (mg/kg p.o.)	Behavioral measure: Response speed	Behavioral measure: Choice accuracy	Behavioral measure: Response omission	Reference
Scopolamine	Aniracetam	30	↑	⇧	⇩	56
	N-anisoyl-GABA	30		⇧	⇩	
	p-Anisic acid	30		⇧	↓	
Apomorphine	Aniracetam	10	⇧	⇧	⇩	57
	N-anisoyl-GABA	10		⇧	⇩	
	2-Pyrrolidinone	10, 30		⇧	⇩	
8-OH-DPAT	Aniracetam	10, 30	⇧	⇧	⇩	52
	N-anisoyl-GABA	30		⇧	⇩	
	2-Pyrrolidinone	10	⇧			
DOI	Aniracetam	10	⇧			52

⇧ or ⇩: a significant ($P < 0.05$) increase or decrease; ↑ or ↓: tendency to increase or decrease.

choline (ACh) receptor antagonist, reduced response speed and choice accuracy and increased response failures, as evidenced by prolonged choice reaction time, decreased percentage of correct and increased percentage of no response, respectively (56). These behavioral changes in the operant task suggest attention deficit and a reduced arousal or vigilance. By systemic administration aniracetam, 10–100 mg/kg p.o., reversed all of the scopolamine-induced performance impairments, most effectively at 30 mg/kg p.o. (Table 1). The *in vivo* metabolites of aniracetam, *N*-anisoyl-GABA and *p*-anisic acid, mimicked the effects of aniracetam in the scopolamine model. Apomorphine, 0.1 mg/kg s.c., a mixed dopamine (DA) D_1 and D_2 receptor agonist, similarly impaired the response speed, choice accuracy and response failures in this task, while aniracetam, 10–100 mg/kg p.o., restored the apomorphine-induced poor performance, most effectively at 10 mg/kg p.o. (Table 1) (57). *N*-anisoyl-GABA and 2-pyrrolidinone, another metabolite of aniracetam, have similar effects. Nabeshima, et al. (49) found that aniracetam was also effective on the apomorphine-induced attention loss in a water maze task. 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), 0.3 mg/kg s.c., a selective 5-HT_{1A} receptor agonist, induced the same impairments as scopolamine and apomorphine, while aniracetam, 10–100 mg/kg p.o., ameliorated all of the 8-OH-DPAT-induced performance deficits with a maximal effect at 30 mg/kg p.o. (Table 1) (52). *N*-anisoyl-GABA and 2-pyrrolidinone exhibited aniracetam-like effects. d1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a selective 5-$HT_{2A/2C}$ receptor agonist, aggravated all the behavioral responses at 3 mg/kg s.c. but only slowed the response speed at 1 mg/kg s.c., suggesting a preferential deficit of selective attention (52). Aniracetam, 3 to 30 mg/kg p.o, improved the slow response speed induced by DOI, 1 mg/kg s.c., most effectively at 10 mg/kg p.o., whereas the three major metabolites were ineffective in this model (Table 1) (52). In another study, aniracetam, 10–100 mg/kg p.o., dose-dependently attenuated DOI-induced head-twitch response in rats (88). The agents used to induce the routine performance impairment in the choice reaction task did not affect motor activity or food motivation in rats under test con-

ditions and aniracetam not only modified motor activity but also caused motivational change in the presence or absence of these chemical inducers. Thus, these results indicate that cholinergic, dopaminergic and serotonergic systems in the brain are involved in the regulation of attention and vigilance functions and that aniracetam may contribute to amelioration of the functional perturbation by interacting with the damaged nervous system or the related neuronal circuits.

Impulsiveness

Clinical impulsivity often appears as aggression, violence, agitation, self-destruction, violent suicidal or homicidal behavior and addiction in many neuropsychiatric disorders, such as attention-deficit/hyperactivity disorder (ADHD), compulsive personality disorder, obsessive-compulsive disorder, bulimia and alcoholism (2,87). The underlying mechanism may conceivably involve serotonergic or serotonergic-dopaminergic interactions (20,21,87).

The two-lever choice reaction task is also useful for examining impulsive behavior in well-trained rats (56). Intracerebroventricular injection of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) (1009 ng/rat), an AMPA receptor antagonist, caused a selective increase in premature response, which was used as an index of impulsivity, without any modification in response speed and choice accuracy in the task, suggesting that NBQX affects only impulsivity without altering attentional and vigilance processing, memory function, food motivation and motor ability (55). Aniracetam, 30 mg/kg p.o., a positive allosteric modulator of AMPA receptors (14,34,42), as well as AMPA (55.9 ng/rat), completely restored the NBQX-induced impulsivity, probably by recovering AMPAergic dysfunction. These results indicate that central AMPA receptors are tonically involved in the regulation of impulsivity and aniracetam acts *in vivo* as an AMPA receptor modulator. The modulation of AMPA receptors by aniracetam has been previously shown to alter the anticonvulsant action of NBQX or contextual learning impairment in DBA/2 mice (6,39). It is conceivable that the anatomical substrates involved in the effects of aniracetam may be localized in the mesocortical serotonergic pathway. In another study, aniracetam, 1 to 30 mg/kg p.o., dose-dependently improved the methamphetamine-induced preferential increase in impulsive lever pressing of rats during the periods of anticipation and perseveration in the choice reaction task (25).

Hyperactivity

ADHD is characterized by three major clinical features: inattention, hyperactivity and impulsiveness (102). In addition, ADHD is frequently accompanied by comorbid disorders, such as anxiety, depression, dysthymia, aggression, sleep disorders, learning disability, conduct disorder, and tics or Tourette's disorder (86,102).

Spontaneously hypertensive rat (SHR) is thought to be the best-validated rat model of ADHD (76), as characterized by hyperactivity, deficient sustained attention and motor/cognitive impulsiveness (15,76,99). In an open-field test, SHR indeed exhibited higher ambulatory (locomotor activity and line crossing) and exploratory (rearing) activities than their normotensive control, Wistar Kyoto rat (WKY). These effects were seen in young (4 weeks old) and adult (20 weeks old) rats (unpublished data by K. Nakamura and Y. Tanaka). Acute administration of aniracetam, 30 and 100 mg/kg p.o., tended to sup-

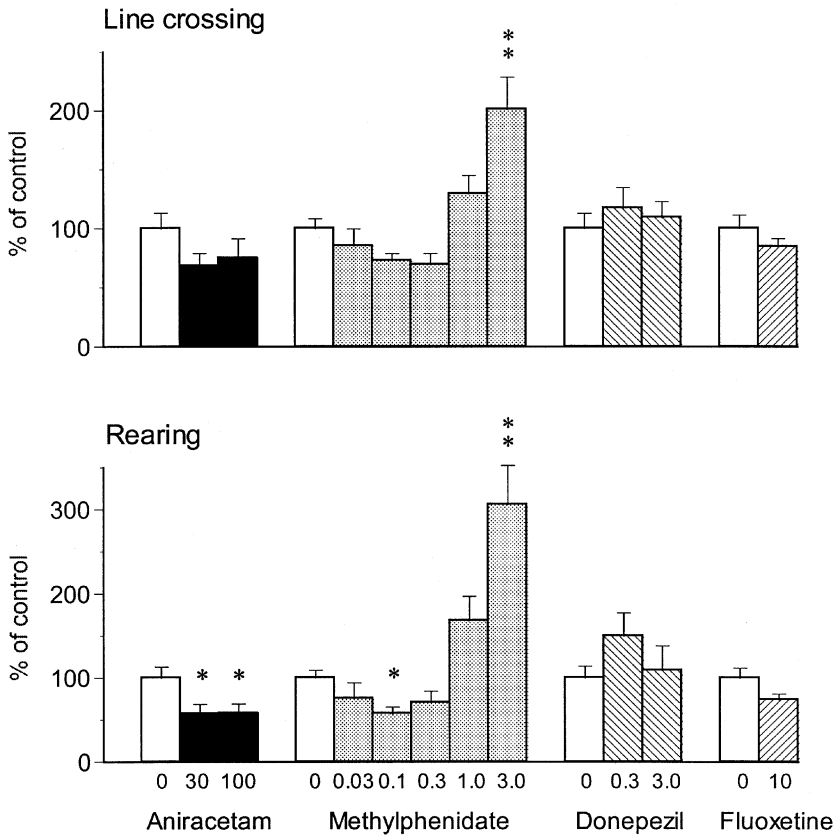

Fig. 1. Effects of repeated administration of aniracetam, methylphenidate, donepezil and fluoxetine on line crossing and rearing of young SHR. Compounds (mg/kg) or vehicle were orally administered once daily for 5 consecutive days and an open-field test was performed for 30 min at 1 h after the last dosage. The behavior was analyzed during the last 15 min. Data, calculated as percentage of vehicle control values, show means ± S.E.M. (n = 6 to 15 rats per group). *$P < 0.05$ and **$P < 0.01$ vs. corresponding control.

press those behavioral abnormalities in young SHR and significantly decreased rearing in adult SHR. Repeated dosages of aniracetam, 30 and 100 mg/kg p.o., once daily for 5 days, augmented the ameliorating effects of the single dose in rats of either age (see Fig. 1 for young SHR). No adverse effects, such as tics induction, appetite suppression or general sympathomimetic stimulation, were produced by aniracetam at a single or repeated doses. Methylphenidate, a psychostimulant, showed a dual action in young SHR: amelioration of behavioral abnormalities at lower doses (0.03–0.3 mg/kg p.o.) and aggravation at higher doses (1 and 3 mg/kg p.o.); these effects were potentiated by repeated dosages (Fig. 1). Donepezil, 0.3 and 3 mg/kg p.o., an acetylcholinesterase inhibitor, and fluoxetine, 10 mg/kg p.o., a 5-HT reuptake inhibitor, which are now under development for the treatment of ADHD (11,86), were efficacious mainly in adult SHR. There is accumulating evidence indicating abnormal neurotransmission in the brain of SHR, including its sub-strain, stroke-prone SHR. The behavioral improvement by aniracetam may result from the functional acceleration of one or more of the neurotransmitter systems in the brain. In another study,

subacute administration of aniracetam, 30 mg/kg i.p., once daily for 3 days, effectively attenuated the severity of abnormal habituation and hyperactivity detected at three months after a 10-min forebrain ischemia in gerbils (44).

Fear and Anxiety

Impaired or abnormal neurotransmission, especially of 5-HT, in the brain is implicated in the neurobiology of various types of anxiety. Aniracetam had been reported to have no anxiolytic-like action in modified Vogel and Geller-Seifter conflict tests in rats (42, unpublished data by J.R. Martin). However, aniracetam has been recently shown to produce a wide range of anxiolytic effects in three different mouse models of anxiety: social interaction, elevated plus-maze and conditioned fear stress (54).

The social interaction test is a useful animal model for evaluating anxiolytic compounds, which are used in the treatment of social phobia, social failure/impairments and emotional immaturity. Known anxiolytics, antidepressants, several types of stimulators and inhibitors were tested in this model under the high light (380 lux) and unfamiliar condition (13). The anxiety produced by social contact appeared to depend on not only serotonergic but also dopaminergic and cholinergic mechanisms. Systemic administration of aniracetam, 10 to 100 mg/kg p.o., increased total interaction time and total interaction frequency for 5 min at all tested doses. The anxiolytic effects resembled those of the combined treatment with *N*-anisoyl-GABA, 2 mg/kg p.o., and *p*-anisic acid, 10 mg/kg p.o., or with *N*-anisoyl-GABA, 2 mg/kg p.o., and 2-pyrrolidinone, 10 mg/kg p.o. Detailed analysis of the total interaction time of each social behavior revealed that aniracetam exhibited substantial effects on trunk sniffing and following and also tended to increase the other social behaviors (facing, neck/tail licking and genital investigation). The anxiolytic effects of aniracetam were completely blocked by haloperidol at a non-sedative, non-cataleptic dose of 0.03 mg/kg i.p.; this complete blockade was observed against all types of social behaviors. Mecamylamine, 1 mg/kg s.c., a nicotinic ACh (nACh) receptor antagonist, markedly inhibited the effects of aniracetam, reflecting the complete reversal especially of sniffing and facing. Ketanserin, 0.3 mg/kg i.p., a preferential 5-HT_{2A} receptor antagonist, almost blocked the effects, as evidenced by a marked decrease in licking, sniffing and following. Haloperidol and mecamylamine, by themselves, at the doses used, had no effect on total social interaction time or locomotor activity, whereas ketanserin slightly reduced the social interaction score and locomotion. These results indicate that the effects of aniracetam are mediated by an interaction between three neuronal systems (cholinergic, serotonergic and dopaminergic).

The elevated plus-maze test is thought to produce unconditioned fear (clinically compatible with panic anxiety) by single exposure to open spaces (19). Aniracetam was tested using a previously described method (38); it had also anxiolytic activity. By systemic administration aniracetam, 10 to 100 mg/kg p.o., increased the percentage of entries into the open arms in a dose-dependent manner and at 30 mg/kg also the percentage of time spent on the open arms. Among its major metabolites, only *p*-anisic acid, 30 mg/kg p.o., had similar anxiolytic effects. Comparative studies with different classes of compounds suggest that cholinergic and serotonergic mechanisms may contribute to the anxiolytic effects of aniracetam.

Conditioned fear stress-induced freezing behavior is regarded as an animal model of anticipatory/generalized anxiety and also of panic disorder (5,27). In this model (3), anira

cetam, 10 to 100 mg/kg p.o., shortened the freezing time during the observation period of 6 min most effectively at 10 mg/kg p.o. and concurrently increased locomotion in the test chamber. These effects were not due to a general motor activation, since the locomotor activity in the chamber of mice, naive to electric footshock, was unaffected by aniracetam. *N*-anisoyl-GABA, 30 mg/kg p.o., reproduced the anxiolytic effects of aniracetam. From the results with other known compounds, examined in the model, the conditioned fear stress-induced freezing behavior and the antifreezing effects of aniracetam appear to be mediated mainly by both, serotonergic and dopaminergic mechanisms.

Depression

No studies have been reported that experimentally evaluated the effects of aniracetam on depression or a depressed state. Antidepressant-like effects were examined in a forced swim test as one of the behavioral despair tests (72), which is sensitive to and relatively specific for clinically available antidepressants and, therefore, a useful animal model for evaluating potential antidepressants (72,73).

By subacute administration (3 doses over 2 days) to 25 to 30 months old rats aniracetam, 100 mg/kg p.o., markedly reduced immobility time during a 5-min swim on day 2 without any visible modification of behavior (60). However, in 9 weeks old rats aniracetam, 10 to 100 mg/kg p.o. (3 doses over 2 days), failed to decrease immobility time. By chronic administration at 100 mg/kg p.o., once daily for 14 days, aniracetam, slightly but significantly, reduced immobility time. Combined subacute treatment with 2-pyrrolidinone, 50 mg/kg p.o., and *N*-anisoyl-GABA, 10 mg/kg p.o., had solely an antifreezing effect in aged rats; aniracetam had similar effect. The subacute antidepressant-like effects of aniracetam in aged rats were potentiated by singly administered scopolamine, 0.03 mg/kg i.p. In contrast, the effects of aniracetam were completely reversed by mecamylamine, 10 mg/kg i.p., or haloperidol, 0.1 mg/kg i.p., but were not affected by ketanserin, 1 mg/kg i.p. Scopolamine, 001 to 0.1 mg/kg i.p., alone, dose-dependently reduced immobility time, while other receptor antagonists alone had no significant effect on this parameter. The authors suggested that in the forced swim test aniracetam, like most known antidepressants, is more effective in animals with brain dysfunction, especially in those in hypodopaminergic state, that occurs in the elderly (47). Since cholinergic and dopaminergic mechanisms appear to be involved in the pathophysiology of depression (4,8,41), aniracetam may exhibit its antidepressant-like property by facilitating both, cholinergic and dopaminergic systems in the brain.

REM Sleep Impairment

Effects of aniracetam on basic sleep patterns and sleep-wakefulness rhythm were examined with a polysomnogram recorded through electroencephalographic and electromyographic electrodes in stroke-prone SHR (SHRSP) (31). SHRSP are considered a good animal model of multiple cerebral infarcts (77) and have central cholinergic and dopaminergic deficits (58,59,84,91).

Thirteen weeks old SHRSP, loaded with 1% saline for 5 weeks, showed the same typical nocturnal behavioral pattern as age-matched WKY. However, SHRSP had a shorter rapid-eye movement (REM) sleep during the light period and a longer non-REM sleep during the dark period than WKY. Aniracetam, 15 and 50 mg/kg p.o., was adminis-

tered twice daily for 5 consecutive days (days 1 to 5). At 30 mg/kg p.o./day it increased the reduced diurnal REM sleep on day 5, while at 100 mg/kg p.o./day aniracetam tended to increase REM sleep. The improvement was due to an increase in the number of episodes and not in the duration of episodes. Aniracetam, at either dose did not affect elevated nocturnal non-REM sleep and at a single dose had no effect on the sleep parameters on day 1. These results indicate that aniracetam is effective in alleviating the disturbed sleep-wakefulness rhythm by ameliorating the suppressed REM sleep.

Disturbed Temporal Regulation

CNS disorders or aging often cause chronobiological impairment (45,98). In elderly, as well as in patients with CNS disorders, the functional loss of the circadian clock or timekeeping system would appear as sleep disorder, circadian rhythm disorder, nocturnal behavioral problems, and/or decreased activity in daily living (ADL). Mealtime-associated circadian anticipatory behavior, a useful animal model of temporally regulated behaviors or circadian time keeping, is diminished in aging rats (46,82).

The general experimental design was essentially the same as reported previously (82). Anticipatory behavior emerged on day 7 in young rats when the animals were fed only at a fixed time for 6 days, while in the aging rats the activity was diminished. By repeated administration (once daily for 7 days) aniracetam, 30 or 100 mg/kg p.o., restored the diminished anticipatory behavior in aging rats in a dose-dependent manner, as did physostigmine, 0.1 mg/kg s.c., an acetylcholinesterase inhibitor (89). The ameliorating effects of aniracetam were independent of a change in appetite, motor ability or circadian motor activity rhythm. Moreover, recent study showed significant attenuation of the effects of aniracetam by either scopolamine, 0.1 mg/kg i.p., or haloperidol, 0.1 mg/kg i.p. (90). Thus, aniracetam may restore the aging-diminished circadian anticipation (probably due to dysfunction of food-entrainable oscillator system) by facilitating cholinergic and dopaminergic neurotransmissions in the brain.

On the other hand, in darkness aniracetam, 10 to 100 mg/kg p.o., dose-dependently potentiated the light pulse-induced phase delay in hamsters running in a wheel. At 100 mg/kg p.o. aniracetam also facilitated the light-elicited c-Fos expression in the suprachiasmatic nucleus, suggesting the acceleration of the photic entrainment of the circadian clock by aniracetam (48). However, this effect was not associated with an increased expression of mammalian clock genes, mPer1 and mPer2, in the nucleus (personal communication with T. Moriya).

Poor Performance

Hypobulia, malaise, motivation loss, and lack of spontaneity occurring as a consequence of CNS disorders greatly reduce ADL in patients and markedly lowers their quality of life. The two-lever choice reaction task performed by rats is based on a food-reinforced (motivated) operant behavior. The disruption or progressive decline of habitual task performance, induced by food satiation in this task is, therefore, thought to be useful as an index of motivation drive. Aniracetam was evaluated in this model using food-restricted rats that stably maintained excellent task performance over 1 year (53).

Successive free feeding for about 2 months greatly diminished the performance of aged rats, as revealed by a reduction in lever pressing with low choice accuracy, high choice

omission and slow response speed. By chronic administration to these animals aniracetam, 10 and 30 mg/kg p.o., once daily for 14 consecutive days, dose-dependently restored the satiation-induced poor task performance, especially the rate of lever pressing, choice accuracy and no choice, without altering task-associated motor activity, premature response (impulsivity) or body weight, suggesting no change in motor ability, food motivation or reference memory (89). The authors indicate that satiation satisfies the appetite of rats and reduces their motivation to perform and to attain the operant task. Aniracetam may enhance voluntary activity by facilitating the driving force or motivation to achieve the instrumental task, or by improving daily attention and vigilance failure (52,56,57).

Bladder Overactivity

The pontine micturition center is modulated by the cortical-diencephalic pathways via ACh and GABA as inhibitory and DA and glutamate (Glu) as excitatory transmitters (7,63). General reduction in cerebral function with aging or brain damage leads, therefore, to an increase in urinary frequency and incontinence. Effects of aniracetam on neurogenic overactivity of the urinary bladder were examined in rats subjected to occlusion of left middle cerebral artery (51).

By acute administration aniracetam, 30 to 300 mg/kg p.o., or 0.025–2.5 μg/rat i.c.v., dose-dependently increased bladder capacity of rats without affecting infarction volume in the ischemic brain region and had no effect in sham-operated rats. Pretreatment with atropine, 1 μg/rat i.c.v., a muscarinic ACh receptor antagonist, completely antagonized aniracetam, 100 mg/kg p.o.-elicited increase in bladder capacity. The authors suggested that the ameliorating effects of aniracetam may be mediated by activation of a cholinergic inhibitory pathway in the frontal cortex.

PHARMACOKINETICS

Aniracetam is promptly metabolized and rapidly eliminated from circulation (43). In contrast, further metabolism of 2-pyrolidinone and of *p*-anisic acid is generally slow in rats. The pharmacokinetics of a single dose of aniracetam and its major metabolites in the brain of rats was recently described (65).

After single intravenous injection of aniracetam, 30 mg/kg, the drug and its metabolites: 2-pyrrolidinone and *p*-anisic acid, rapidly entered the examined brain regions: cerebral cortex, hippocampus and thalamus. In these three regions the AUC_{0-2} of 2-pyrrolidinone was the highest (9.0–9.3 μg·h/g), followed by *p*-anisic acid and aniracetam, 0.10 to 0.15 μg·h/g. The mean residence time was much higher for 2-pyrrolidinone (0.99 to 1.03 h) than for aniracetam (0.39 to 0.47 h) or *p*-anisic acid (0.68 to 0.71 h), suggesting a rapid decline in the levels of aniracetam or *p*-anisic acid in these regions. High elimination rate constants were consistent with these findings. The sustained levels of 2-pyrrolidinone reflected a slow elimination in plasma. *N*-anisoyl-GABA concentrations were below the detection limit in all regions studied. The AUC_{brain}/AUC_{plasma} ratio of 2-pyrrolidinone was 53 to 55%, whereas those of *p*-anisic acid and aniracetam were 3.9 to 4.2% and 2.4 to 3.2%, respectively (64,65). Oral administration of aniracetam (50 mg/kg) showed concen-

tration-time profiles similar to those after the intravenous injection of aniracetam and *p*-anisic acid, but that of 2-pyrrolidinone was characterized by a rapid elimination. The rank order of concentrations (AUC_{0-4}) was *p*-anisic acid (3.2–3.4 μg·h/g), 2-pyrrolidinone and aniracetam (0.18–0.22 μg·h/g). The AUC_{brain}/AUC_{plasma} ratio was 21 to 22% for 2-pyrrolidinone and 10 to 11% for *p*-anisic acid.

MECHANISMS OF ACTION

It has so far been reported that aniracetam enhances synaptic efficacy by facilitating long-term potentiation and activated energy metabolism by increasing ATP production (34,42). Additionally, the drug was suggested to be a positive modulator of cholinergic and glutamatergic neurotransmission. Recent biochemical findings that are relevant to the mechanism(s) of action of aniracetam are summarized below.

Cholinergic Mechanism

In *in vivo* microdialysis studies, aniracetam, 100 mg/kg p.o., enhanced ACh release in the hippocampus, but not in the parietal cortex, of freely moving rats (16). When, however, aniracetam, 1 μM, was infused locally into the cholinergic nerve terminals, nucleus reticularis thalami, dorsal hippocampus or prefrontal cortex (PFC) of freely moving SHRSP, characterized by a central cholinergic deficit (58,84,91), aniracetam failed to increase ACh release in these regions (58). Additionally, aniracetam, 1 nmol, by direct injection into the ACh cell body in the pedunculopontine tegmental nucleus (PPTg) of SHRSP, had no effect on ACh release in the nucleus reticularis thalami, which receives cholinergic projections from the PPTg. In contrast, local perfusion with aniracetam metabolites, *N*-anisoyl-GABA (0.1 and 1 μM) and *p*-anisic acid (1 μM), but not 2-pyrrolidinone, enhanced the release with a delayed onset in all the tested regions and the nucleus. Moreover, microinjection of *N*-anisoyl-GABA, 1 nmol, into the PPTg increased ACh release in the nucleus reticularis thalami. Similarly, the local infusion of the metabolite (1 μM) into the laterodorsal tegmental nucleus (LDTg), another of the ACh cell bodies, appeared to increase ACh release in the ventral tegmental area (VTA), which is innervated cholinergically, primarily by the LDTg (85). Moreover, the *N*-anisoyl-GABA-elicited ACh release in the PFC of SHRSP was demonstrated to be mediated by group II mGlu receptors but not by group I mGlu or AMPA receptors (84). The authors suggested that aniracetam may facilitate cholinergic neurotransmission throughout the brain, probably via group II mGlu receptors activated by its metabolites, *N*-anisoyl-GABA, and possibly by *p*-anisic acid. Another publication reported that aniracetam, at very low concentrations, 0.1 to 10 nM, potently and consistently augmented α4β2-type ACh currents and at 10 μM weakly inhibited α7-type currents in rat cortical neurons (103). These findings differed from those obtained in *Xenopus laevis* oocytes (61).

Aniracetam, 15 and 50 mg/kg p.o., twice daily for 6 consecutive days, dose-dependently increased choline acetyltransferase (ChAT) activity only in the thalamus among five brain regions of SHRSP (58). In contrast, aniracetam had no effect on ChAT activity in WKY. These results suggested that aniracetam preferentially activates the reticulothalamic cholinergic pathway. By chronic administration (once daily for 28 days) aniracetam,

50 mg/kg p.o., increased ChAT activity in the cerebral cortex but not in the hippocampus or striatum of ischemic aging rats, although it had no effect on the ChAT activity in any of these brain regions *in vitro* (10). In gerbils aniracetam rectified hippocampal cholinergic neurotransmission system that was damaged due to ischemia-induced pyramidal cell death (32). In another study aniracetam, by systemic administration at 50 mg/kg i.p., twice daily for 7 consecutive days, prevented reduction of glucose levels but did not alter the reduced ChAT activity in the frontal cortex of basal forebrain-lesioned rats (66).

Glutamatergic Mechanism

It has been suggested that aniracetam is a dual allosteric modulator of ionotropic AMPA and mGlu receptors (14,18,34,42,70,71). Recent studies further examined the participation of the glutamatergic system in the *in vitro* and *in vivo* effects of aniracetam.

As mentioned above, *N*-anisoyl-GABA and *p*-anisic acid, but not aniracetam itself, indirectly interact with group II mGlu receptors leading to an increase in ACh release (84). However, aniracetam, 0.01 to 1 μM, abolished the antagonism by kynurenic acid of *N*-methyl-D-aspartate (NMDA)-evoked [^{3}H]norepinephrine release from rat hippocampal slices in a concentration-dependent manner. The AMPA-induced [^{3}H]norepinephrine release from brain slices was enhanced by aniracetam, but only at a concentration of 100 μM, suggesting a selective modulation of NMDA receptor function (69). Through the interaction with NMDA, but no ACh receptors, aniracetam, 1 μM, increased population spike amplitude in the rat hippocampal slices, suggesting an induction of a "long-term potentiation-like" facilitation without tetanus stimulation (62). Repeated administration (once daily for 7 days) of aniracetam, 20 mg/kg s.c., to rats upregulated [^{3}H]ifenprodil and [^{3}H]MK-801 binding and decreased NR2B mRNA levels, one of the NMDA receptor subunits, in the hippocampus (24), and also reduced 5-HT_6 receptor mRNA levels in the striatum, possibly mediated by an AMPA receptor desensitization mechanism in the dorsal raphe nucleus (DRN) (23). While aniracetam and its several metabolites, at a concentrations up to 10 μM, did not directly interact with the excitatory amino acid receptors (42), specific [^{3}H]aniracetam binding to rat brain membranes is observed, most probably to AMPA receptors (12).

Systemic injection of 7-nitroindazole (10 mg/kg i.p.), a preferential inhibitor of neuronal nitric oxide (NO) synthase, impaired a step-through passive avoidance task in mice (Fig. 2). In this test, the post-training treatment with aniracetam, 10, 30 or 100 mg/kg p.o., ameliorated the retention latency. 2-Pyrrolidinone, 10 mg/kg p.o., mimicked the effects of aniracetam. Co-injection of l-arginine, 200 and 500 mg/kg i.p., a NO donor, with 7-nitroindazole reversed the acquisition (learning) deficits. The fact that aniracetam activated the brain NO system is consistent with the observations that at 100 mg/kg p.o., it enhanced Glu release and tended to increase oxidative NO metabolites in the PFC of freely moving SHRSP with a reduced Glu release (92). In contrast, aniracetam had no effect on GABA release *in vitro* (96) or *in vivo* (92). The aniracetam-induced Glu release may be achieved by aniracetam itself via several mechanisms, such as stimulation of NMDA receptors (29,69) or activation of nACh receptors by released ACh (81,93,103).

It is conceivable that after systemic administration aniracetam, and/or its metabolites, reach the brain at low concentrations, enhance Glu release and indirectly activate diverse Glu receptors (NMDA, AMPA and mGlu receptors). This activation leads to ACh release and NMDA receptor-mediated NO generation (29). However, the observation that micro-

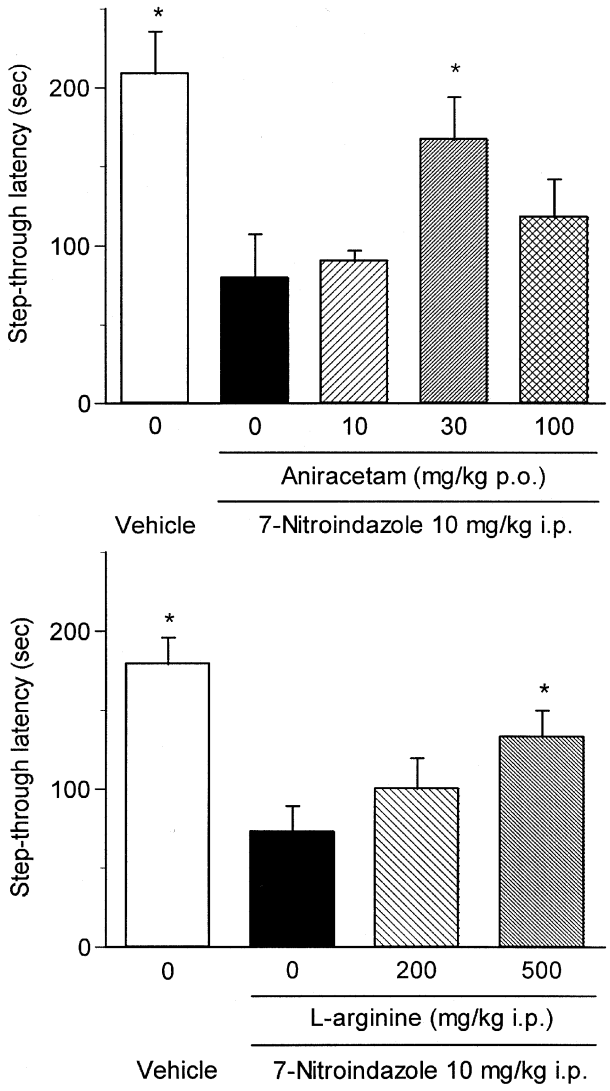

Fig. 2. Effects of aniracetam and l-arginine on 7-nitroindazole-impaired retention latency in the step-through passive avoidance task. Mice (n = 17 to 20 per group) were injected with 7-nitroindazole or vehicle 30 min prior to the training session. Aniracetam was administered immediately after the training session and l-arginine was given concomitantly with 7-nitroindazole. Data show means ± S.E.M. *$P < 0.01$ vs. 7-nitroindazole alone.

injection of aniracetam into the PPTg and its local infusion into the nerve terminal regions had no effect on ACh release, suggests no direct interaction with the Glu receptors (58). Nevertheless, at nanomolar concentrations aniracetam might show possible association with NMDA receptors, as revealed by the previously discussed *in vitro* study (69). Aniracetam might bind to a specific site after opening of ion channels of AMPA receptors by the released Glu (12). This effect may be unrelated to the known electrophysiological effect of aniracetam on AMPA receptors, an effect that requires mM concentrations of the drug, while it is supported by functional *in vivo* studies (6,39).

Monoaminergic Mechanism

In vivo microdialysis technique in freely moving SHRSP was used to ascertain the effects of aniracetam and its metabolites on dopaminergic and serotonergic systems, DA and 5-HT release (59,85).

The studies demonstrated first a uniform deficit in dopaminergic neurotransmission in the nigrostriatal and mesocorticolimbic pathways in SHRSP, as evidenced by a reduced DA release in various brain regions (dorsal hippocampus, basolateral amygdala, nucleus accumbens shell, striatum, and PFC) (59). By systemic administration aniracetam 30 and 100 mg/kg p.o., dose-dependently and selectively increased the extracellular levels of DA, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-HT in the dorsal hippocampus, basolateral amygdala and PFC of SHRSP (59). The aniracetam-sensitive regions appear to play an essential role in the regulation of emotion and mood, motivation, sleep-wakefulness and cognition with anatomical and functional connections among those regions (35,36). Therefore, the site-specific activation in the mesocorticolimbic dopaminergic and serotonergic pathways strongly suggests possible therapeutic use of aniracetam in the treatment of neuropsychiatric disorders caused by psychological and physical stress and stimuli.

At 100 mg/kg p.o. aniracetam elicited DA and 5-HT release in the PFC. This effect was mimicked solely by local infusion of *N*-anisoyl-GABA,1 μM, into the VTA and DRN, respectively (85). The effects of oral aniracetam and local *N*-anisoyl-GABA on DA and 5-HT release were completely blocked by local perfusion of mecamylamine, 100 μM, into the same areas. Additionally, *p*-anisic acid, 1 and 10 μM, enhanced DA release and *N*-anisoyl-GABA, 0.1 and 1 μM, increased 5-HT release in the same region when they were infused into the PFC, while aniracetam, 1 μM, had no effect. Therefore, in contrast to previous speculations (23), the authors deny possible involvement of AMPA receptors not only in the VTA and DRN, but also in the terminal regions. Furthermore, these findings indicate that *N*-anisoyl-GABA and *p*-anisic acid are responsible for the monoamine release elicited by orally administered aniracetam. They also suggest that the effects of *N*-anisoyl-GABA may be mediated by nACh receptors (α3β4-subtype and possibly α4β2-subtype but not α subtype) (1,103) and by NMDA receptors (67) in the VTA and DRN.

Modulation of Neural Circuits

The mechanisms involved in neurotransmitter release elicited by aniracetam given systemically are multiple, since aniracetam has at least three different target sites, such as cholinergic cells, monoaminergic cells and their projection terminals. In addition to simple cholinergic–monoaminergic interaction, there appears to be a cholinergic–glutamatergic–monoaminergic or glutamatergic–monoaminergic interaction. Moreover, serotonergic–dopaminergic interaction between the DRN and VTA may be involved. The mutual association among these neurotransmitters appears to form highly complex neural circuits.

It is unclear whether aniracetam elicits a concomitant release of Glu and monoamines in their nerve terminals or whether Glu release precedes as a trigger for monoamine release. One possible explanation is illustrated in Fig. 3. It may be speculated that aniracetam (or its multiple metabolites) enhances ACh release from the cholinergic nerve terminals throughout the brain by stimulating the ACh cell bodies, the PPTg and LDTg, or presynaptic mGlu receptors on the terminals (58,84). The released ACh successively sti-

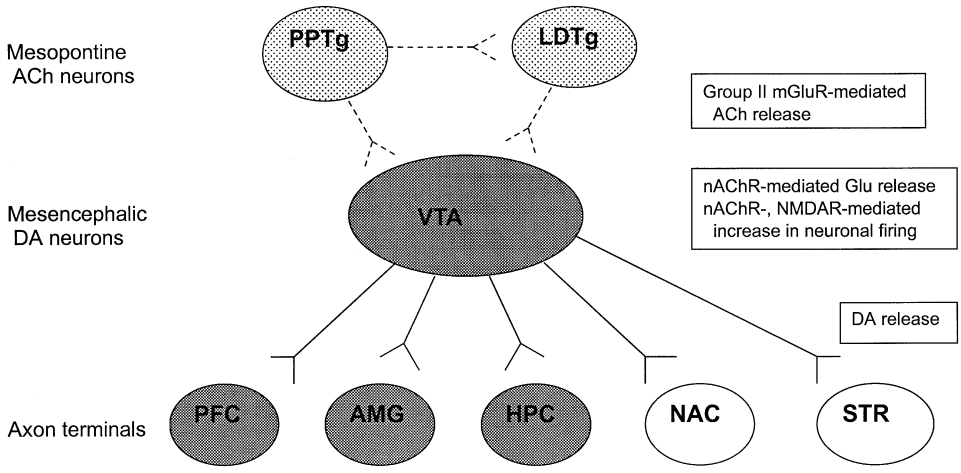

Fig. 3. Regionally specific DA release by aniracetam in mesocorticolimbic pathway and its possible mechanisms of action. Dotted and solid lines show cholinergic and dopaminergic axons, respectively, and shaded ellipses represent the target sites of aniracetam. AMG: amygdala, HPC: hippocampus, LDTg: laterodorsal tegmental nucleus, NAC: nucleus accumbens, PFC: prefrontal cortex, PPTg: pedunculopontine tegmental nucleus, STR: striatum, VTA: ventral tegmental area. Adapted and modified from reference 59.

mulates presynaptic nACh receptors on glutamatergic afferents in the VTA and also somatodendritic nACh receptors on DA cells, leading to Glu release in the VTA and DA release from the dopaminergic nerve terminals, respectively (59,81,85). The putative rise in Glu levels in the VTA then activates somatodendritic NMDA receptors on DA cells and subsequently increases DA release at the mesocorticolimbic dopaminergic terminals. Similar mechanisms may be involved in the enhanced 5-HT release (mesopontine cholinergic–mesencephalic glutamatergic and serotonergic interactions via nACh and NMDA receptors). The released Glu may augment the modulating action of aniracetam on AMPA and NMDA receptors. With respect to the ACh release, elicited by aniracetam, the released Glu appears to trigger the release by stimulating group II mGlu receptors (84) and/or NMDA receptors (24,69).

Signal Transduction

The intracellular mechanisms by which aniracetam enhances neurotransmitter release and accelerates synaptic efficacy have not been fully identified. However, there are some reports regarding the effects of aniracetam on intracellular transducing systems.

Chronic treatment of rats with aniracetam, 50 mg/kg i.p., once daily for 15 days, potentiated both, basal and stimulated (carbachol, DA and norepinephrine, but not forskolin) adenylyl cyclase activity in the frontocortical and hippocampal synaptic membranes (96). Consistent with this, Pizzi et al. (71) reported that aniracetam, 1 μM, had no effect on forskolin-stimulated cyclic AMP formation in rat cerebellar granule cells. In another work, a dose-dependent potentiation of both, basal and stimulated (Gpp(NH)p, forskolin and Mn^{2+}) adenylyl cyclase activity was observed in rat striatum after chronic treatment with aniracetam, 10 and 30 mg/kg i.p., once daily for 3 weeks (95).

Aniracetam, 50 mg/kg i.p., once daily for 15 days, also augmented inositol phosphate production stimulated by angiotensin II in the rat frontocortical synaptosomes (95). At 5 μM it also potentiated quisqualate-induced inositol phosphate formation in the rat cerebellar granule cells (71). Similarly, aniracetam promoted membrane translocation of protein kinase C both *in vitro* (200 nM) and *in vivo* (30 mg/kg p.o.) in the hippocampus of rats but not in the cerebral cortex (40). At 0.1 to 10 μM aniracetam in a concentration-dependent manner facilitated the function of high voltage-gated neuronal Ca^{2+} channels in neuroblastoma-glioma hybrid (NG108-15) cells (101). At 50 mg/kg i.p., once daily for 15 days, aniracetam caused an increase of both, basal and K^+-induced, Ca^{2+} concentrations, in the hippocampal synaptosomes of rats (44).

Aniracetam has been reported to have a neurotrophic action. In mouse cerebellar granule cells cultivated for 18 days in low K^+-containing medium, aniracetam, 1 to 100 μM, not only increased survival but also promoted neurite extension with a maximal effect at 10 μM (14). Aniracetam, 0.01–100 μM, also enhanced nerve growth factor-induced neurite outgrowth in PC12 cells in a concentration-dependent manner (100). In contrast, attenuating effects of brain-derived neurotrophic factor on synaptic fatigue induced by high-frequency stimulation in rat hippocampal CA1 synapses was unaltered by the blockade of AMPA receptor desensitization by aniracetam, 5 mM (17).

POTENTIAL CLINICAL INDICATIONS

Past clinical studies had proven the efficacy and safety of aniracetam in treating emotional disturbances, sleep disorders, behavioral problems, and cognitive impairment in patients with cerebrovascular or Alzheimer's disease (28,31,34,83,94). Recent preclinical studies identified novel beneficial properties of aniracetam, that are related to functional recovery in various animal models of cerebral dysfunctional disorders. Those include hypoattention, hypovigilance-arousal (52,56,57), impulsive behavior (55), hyperactivity, fear and anxiety (54), depression (60), suppressed REM sleep (31), motivational drive reduction (53), chronobiological impairment (89), and bladder overactivity (51).

Based on these results, new potential indications for aniracetam or its metabolites were proposed. Aniracetam may conceivably be indicated in the treatment of delirium, personality (compulsive and borderline personality disorders), and anxiety disorders (obsessive compulsive, social anxiety, panic, and generalized anxiety). It can also be useful in the treatment of depression, social withdrawal and poor social interaction, negative symptoms of schizophrenia, post-traumatic stress disorder, autism, sleep disorders (insomnia and circadian rhythm sleep disorder), chronic fatigue syndrome, and urinary incontinence. Some of the behavioral and psychological symptoms of dementia, observed in the elderly and in patients with CNS disorders, such as disturbed consciousness, reduced spontaneity and motivation, decreased concentration, mental fatigue, nocturnal wandering, and diurnal rhythm disturbances, may be reduced by aniracetam. Additionally, aniracetam was reported to improve neuropsychiatric symptoms as well as intellectual and motor functions in patients with Parkinson's disease and progressive supranuclear palsy (22,26,50). Finally, preliminary study revealed the usefulness of aniracetam in the treatment of vertigo or dizziness associated with cerebral infarction (30) and in the treatment of social with-

drawal as well as language and communication problems in infantile autism. Aniracetam could be effective in the treatment of three major symptoms (inattention, hyperactivity and impulsiveness) of ADHD as well as its comorbid symptoms (anxiety, depression, dysthymia, social failure/impairments, and sleep disorders). Aniracetam may also be useful in increasing ADL and improving quality of life of elderly patients.

SUMMARY

Aniracetam has been developed as a cognition enhancer and used to treat cognitive disturbances, mood and motor performance in patients with stroke and Alzheimer's disease. However, contrary to expectations, its practical benefits in memory impairment were small. Recently, new pharmacological studies expanded its clinical indications. New indications are based on findings that aniracetam restores various types of mental function impairment or cerebral dysfunction in animal models of CNS disorders. These findings suggest possible usefulness of aniracetam in the treatment of personality disorders, anxiety, depression, post-traumatic stress disorder, ADHD, autism, negative symptoms of schizophrenia, and sleep disorders. Clinical trials are needed to validate these new indications.

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