Influence of Aminooxyacetic Acid, a γ-Aminobutyrate Transaminase Inhibitor, on Hereditary Spastic Defect in the Mouse.* (27245)

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γ-Aminobutyric acid (γ-ABA) is of interest in mammalian neurophysiology because large amounts are easily extracted from brain, spinal cord, and retina, as compared to other organs, and because γ-ABA, or a metabolite thereof, may have an important inhibitory function in the central nervous system (CNS)(1). The unique aspect of γ-ABA metabolism in the CNS is the occurrence there of L-glutamic acid decarboxylase, the enzyme which catalyzes the formation of γ-ABA. This enzyme has not been shown to be present in any vertebrate organ other than the CNS. Removal of γ-ABA by transamination with α-ketoglutarate, however, does take place elsewhere as well as in the CNS. γ-ABA levels in the CNS probably are largely a function of the relative rates of formation and utilization by the decarboxylase and transaminase pathways, respectively.

Two carbonyl reagents, hydroxylamine (NH_2OH)(2) and aminooxyacetic acid (AOAA)(3), inhibit activity of γ-ABA transaminase preferentially to glutamic decarboxylase *in vivo* and produce elevations of γ-ABA levels in the CNS(4). Increases in γ-ABA were found in brains of cats, rats, and monkeys after injection of NH_2OH. To date only the mouse has failed to respond to NH_2OH with elevation of γ-ABA in brain, while AOAA was effective in all species studied. The only other change in distribution of freely extractable ninhydrin-reactive constituents in the brains or other tissues of rats treated with these substances was an increased content of β-alanine. β-Alanine-α-ketoglutarate transaminase may be identical with the γ-ABA transaminase. Although most measurements which have been made indicate that elevations of γ-ABA levels are *correlated* with decreases in neuronal excitability, in each instance questions could be raised about the pertinence to the normal animal of the electrophysiological or pharmacological methods employed to test excitability.

Mice with a neuromuscular disorder characterized by spasm were found in a hybrid population by one of us (C.K.C.) at the Jackson Memorial Laboratory about 2 years ago. Breeding tests showed inheritance of this condition to be determined by a single autosomal recessive gene (*spa*) with full penetrance(5). Preliminary histological examination of the central nervous systems failed to show any pathological changes. In the present study it has been possible to show that administration of single doses of AOAA to spastic mice results in prolonged relief of the symptoms.

Materials and methods. Experimental animals. The homozygous spastic mice (*spa/spa*) can be identified at approximately 2 weeks after birth, and the symptoms generally become worse with advance of age. Although some of these animals may have a lifespan close to that of normal littermates, early mortality is high, especially prior to weaning. The spastic mice are difficult to breed and the females are poor mothers possibly because of the intermittent spasms and poor physical condition. These factors have caused a severe limitation in availability of the homozygous animals for experimental studies.

The spastic symptoms have been noted to occur spontaneously in these mice and always can be induced by handling or by some sudden disturbance. The clinical features of varying severity which characterize the homozygous spastic mice are: 1, rapid tremor of relatively large amplitude, particularly prominent in limbs and tail; 2, relative lack

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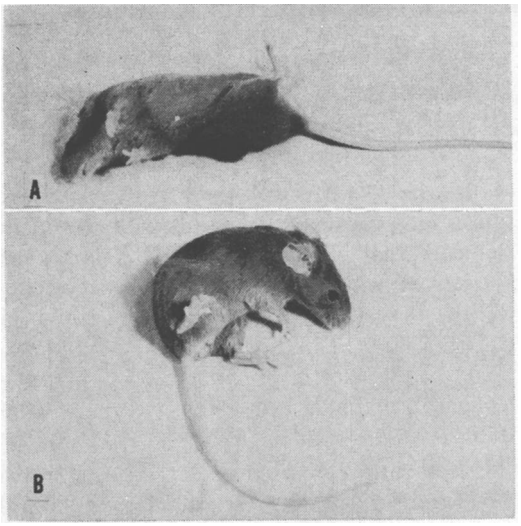

FIG. 1. Typical positions assumed by spastic mice when placed on their backs, as described in text.

of flexibility of trunk movements, with resultant "stiffness" of posture; 3, lack of facility in swimming, despite presence of function of eighth cranial nerve (in contrast to mutant mice of the waltzer-shaker group, which fail to swim and are deaf); and 4, difficulty in righting when placed on back. It should be emphasized that no comparisons have been made as yet with spasticity as manifested in humans with various neurological diseases.

Tests used to assess response to drugs. 1. *Righting time.* A mouse was lifted by the tail from its cage and was placed on a table top. Its behavior was observed for about a minute. Then the base of the tail was grasped and twisted gently but firmly so as to flip the mouse onto its back. The mouse was held by the tail in this position for about one second, then was released; righting time was recorded with a stop watch. Ten successive trials were recorded, with intervals of only a few seconds between trials. A normal mouse or successfully treated spastic mouse regains its feet almost instantaneously and is difficult to place on its back in the first place; righting time in these instances was recorded as "zero seconds." If the mouse did not turn over in one minute, it was placed on its feet by the examiner and the righting time was recorded as "60 seconds." A righting time of one second is abnormal. The difference between one and zero seconds is probably more significant than the difference between 40 and 30 seconds. A spastic mouse placed on its back makes vigorous but inept attempts to right itself. All 4 limbs execute rapid, coarse alternating tremor movements, the tail flails and beats irregularly against the table, and the somewhat stiffened trunk makes feeble twisting or flexing movements inadequate for a righting response(Fig. 1). After many seconds of this behavior, the mouse typically becomes momentarily motionless, then rapidly regains its feet in a single smooth turning motion.

2. *Tremor.* Tremor was evaluated in mice held up by the base of the tail. The tremor was readily felt transmitted through the tail to the examiner's fingers, and was seen in the limbs. Tremor was rated subjectively as "absent", "slight", "moderate", or "severe".

3. *Flexibility.* Subjective assessment was made by placing the mouse on a glass ashtray with smoothly rounded grooves and rims. The ashtray was lifted about 12 inches above the table and was slowly tilted almost vertically. Normal mice climbed about the ashtray for a minute or more and then fell to their feet; spastic mice usually remained stiffly in one position and soon fell onto their backs.

4. *Alertness.* Mice were lifted by the tail and were brought toward an edge or surface. An alert mouse extends its forelimbs in a placing response, while a mouse depressed by disease or overdose of drug makes a feeble placing response or none at all.

Paper chromatography of brain extracts. Three normal adult mice and 4 spastic mice were available for analysis. The mice were killed by cervical dislocation and brains removed rapidly and placed in 80% ethanol. The extract was prepared and paper chromatography performed as described in detail previously(6).

Results. Preliminary tests. Spastic mice were observed after intraperitoneal injection of diphenylhydantoin sodium (Dilantin, parenteral), trimethadione, and AOAA.§ Dilantin in a dose of 50 μg per mouse produced slight improvement only in some mice and tri-

§ Supplied by Dr. M. J. Vander Brook, Upjohn Co., Kalamazoo, Mich.

TABLE I. Effect of Aminooxyacetic Acid (AOAA) on Righting Time of Spastic Mice.

Mouse No.	Pretreatment trials: Individual times (sec.)	Pretreatment trials: Mean	AOAA dose (μg/g)	3 hr post-treatment trials: Individual times (sec.)	3 hr post-treatment trials: Mean
354	3,60,25,60,60,60,60,60,60,60	50.8	5	0,0,0,0,2,0,0,0,0,4	.6
1110	0,0,0,0,0,3,1,2,1,6	1.3	5	0,0,0,0,0,0,0,0,0,0	.0
1125	1,4,35,1,3,19,12,60,3,7	14.5	5	0,0,7,0,25,17,19,1,1,20	9.0
355	7,50,23,7,42,60,20,37,60,52	35.8	10	0,0,4,2,12,6,0,11,3,0	3.8
384	0,5,0,0,1,11,1,60,7,13	9.8	10	3,5,0,0,3,1,0,0,0,3	1.5
387	0,0,6,0,0,0,2,2,0,4	1.4	15	0,0,0,0,0,0,0,0,0,0	.0
388	4,4,4,22,3,31,39,46,8,10	17.1	15	0,0,1,0,4,0,4,0,0,0	.9
1127	30,10,3,6,20,6,2,3,3,2	8.5	15	0,0,0,0,0,0,0,0,0,0	.0

methadione in a 100 μg dose had no perceptible effect on the functions tested. AOAA in doses of 15-30 μg/g body weight was found to be remarkably effective in decreasing the symptoms in the mice tested. The following more detailed studies were then made with AOAA.

Response to AOAA. Eight adult spastic mice with neurological symptoms of varying severity were injected subcutaneously with AOAA, 5 to 15 μg/g body weight. The righting response trials of all animals on a single representative day are recorded in Table I. Each mouse served as its own baseline control before treatment. After treatment, all except No. 1125 showed marked improvement within 3 hours, sustained for 12 to 24 hours or more. Many of the treated animals ran and climbed in a manner indistinguishable from normal on casual inspection. However, all showed minimal signs of neurological disorder on detailed examination. In particular, a slight residual tremor was almost always felt in the examiner's fingers when a treated mouse was lifted by the tail.

In another series of trials, an intraperitoneal dose of 20 μg AOAA per g body weight produced maximum improvement within 30 minutes in each of 4 animals tested (including No. 1125). The full effect was sustained for 24 hours in 3 of 4 animals.

Toxic effects were obtained in some animals on a schedule of subcutaneous injections of AOAA once daily for 4 consecutive days (Table II). The most apparent toxic effects were depression of motor activity and decreased alertness. A depressed animal usually stood in one place and did not explore its en-

TABLE II. Effect of Repeated Injections of Aminooxyacetic Acid on Spastic Mouse No. 355.

Date	Time	AOAA (μg/g)	Avg righting time (sec.)	Tremor	Flexibility and alertness
8/ 9	2:05 p.m.		32.0	Severe	Very poor
8/15	9:35 a.m.		21.6	"	" "
8/18	10:00 a.m.		25.7	Moderate	" "
8/22	10:10 a.m.		35.8	Severe	" "
	11:30 a.m.	10			
	2:45 p.m.		3.8	Slight	Good
8/23	11:05 a.m.		1.8	"	"
	11:30 a.m.	10			
	2:45 p.m.		6.8*	"	Very poor*
8/24	10:40 a.m.		.8	"	Good
	11:30 a.m.	10			
	12:22 p.m.		.0	"	"
8/25	11:00 a.m.		.2	"	"
	11:30 a.m.	10			
	4:00 p.m.		2.1*	"	Poor*
8/28	8:45 a.m.		7.7	Moderate	Good

* Righting time was prolonged and flexibility and alertness decreased because of drug toxicity, as described in text.

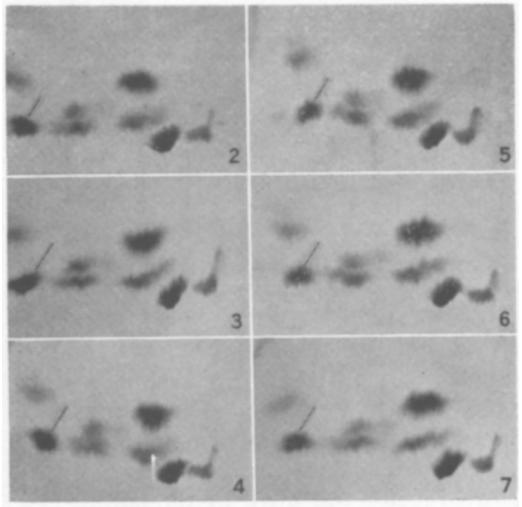

FIG. 2-7. Two-dimensional chromatograms showing virtual identity of distribution of free or easily extractable amino acids in extracts of normal (Fig. 2-4) and spastic (Fig. 5-7) mice. Extracts obtained from 75 mg of fresh weight of tissue were employed for 2-dimensional chromatography (phenol, right to left; lutidine, bottom to top). Arrow points to γ-ABA.

vironment. When placed on its back, it commonly would lie still without showing the rapid flexing and flailing movements characteristic of spastic mice. After a variable number of seconds it would "become aware" of its abnormal posture and right itself with ease. Decreased alertness was confirmed by observation of poor forelimb placing reactions. Some animals, including some depressed ones, showed considerable "jumpiness" when handled. Two of 3 mice receiving 15 μg/g daily, 2 of 2 receiving 10 μg/g, and 0 of 3 receiving 5 μg/g showed clear evidence of toxicity. Toxic signs were evident by the second day of treatment. Four mice, all on the higher AOAA doses, lost 1 to 2 g of body weight per day during the 4-day trial. No mouse gained weight while receiving AOAA.

The paper chromatograms prepared from the extracts of brains of normal and spastic mice were virtually identical (Fig. 2-7), there being no detectable differences between the 2 groups of animals.

Discussion. The experiments reported herein represent the first step in an analysis of the neurological defect found in spastic mutant mice. The clinical tests employed represent a simple, reasonably objective method for the screening of drugs, each animal providing its own baseline control. Detailed neuropathological studies have not yet been undertaken, so that it is not known whether a lesion will be recognized. The paper chromatographic findings make it seem unlikely that a gross abnormality exists in the metabolism of γ-ABA or of the other detectable amino acids. Of course, it is possible that such abnormalities may exist in certain small, restricted areas but that they are masked when analyses of extracts of whole brain are performed. AOAA, at concentrations used in this study, elevates brain levels of γ-ABA(3). The alleviation of symptoms in the spastic mice suggests that the spasticity in the mutants results from an imbalance of excitatory and inhibitory influences in some area of the CNS and that elevated levels of γ-ABA, one of the several inhibitory factors in the CNS, can shift the balance in such a manner as to decrease the interference with normal function. Determination of the extent of elevation of γ-ABA in the various areas of the brains of spastic mice as compared with normal animals, and study of other regional neurochemical parameters, awaits the production of sufficient animals. Meanwhile, it may be suggested that AOAA may find some uses in treatment of some cases of clinical spasticity.

Summary. Experiments were performed on mice with a hereditary neurological disorder characterized by spasm. Simple tests which were used to assess response to drugs included measurement of righting time after being placed on the back, and evaluation of degree of tremor and extent of flexibility and alertness. When single doses of Dilantin, trimethadione, or aminooxyacetic acid were administered intraperitoneally to the spastic mice, only the latter substance was found to be effective in decreasing the symptoms. Subcutaneously injected aminooxyacetic acid in doses of 5 to 15 μg/g produced marked improvement which was sustained for 12-24 hours or more. In some animals which received repeated injections of the drug there was a depression of motor activity, decreased alertness, and loss in weight. Two-dimen-

sional paper chromatograms of extracts of brains of the spastic mice showed no significant alteration of distribution of ninhydrin-reactive constituents by comparison with normal controls.

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Multiplication and Cytopathogenicity of Simian Vacuolating Virus 40 in Cultures of Human Tissues.* (27246)

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The vacuolating simian virus, SV_{40}, was originally found in uninoculated cultures of kidney cells from rhesus and cynomolgus monkeys, in which it multiplies with little or no cytopathic effect(1). Its presence was demonstrated by subinoculation from such systems into cultures of green monkey cells (grivet) in which characteristic cytopathic changes were induced. The agent has subsequently been demonstrated in certain lots of formolized and live attenuated poliovaccines (1,2) as well as in adenovirus vaccine. Eddy and associates showed that injection of fluids from uninoculated rhesus monkey cultures into suckling hamsters was followed on occasion by the development of sarcomas(3). These workers more recently presented evidence that SV_{40} virus in such oncogenic fluids was the responsible factor(4).

Results of serological tests suggest that the virus multiplies in human beings(1,2). Moreover, its intranasal administration to volunteers is reported by certain workers to be followed by respiratory disturbance(5,6). This manifestation was not noted by others(7), although antibody developed in the recipients. Several observations suggest that when given orally to man infection does not occur. Thus SV_{40} was not recovered from stools of patients after oral administration of Sabin poliovaccine containing high concentrations of virus and antibody did not appear in these subjects(1,2). So far attempts to extend the evidence for the capacity of the virus to infect human beings by propagating the agent in primary or stable human tissue cultures have failed. Sweet and Hilleman observed no cytopathic effect in cultures of primary human amnion, stable amnion, Chang liver, Girardi human heart, or HeLa cells(1). Hsiung reported that SV_{40} did not multiply or induce cytopathic changes in cultures of adult human kidney tissue(8) although infectivity was preserved for as long as 58 days (9). In this paper multiplication of SV_{40} associated with characteristic cytopathic effects in primary cultures of human tissues is described.

Materials and methods. SV_{40} Virus. SV_{40} strain VA 45-54 GMK 4, 6/9/61, was obtained from Dr. M. R. Hilleman. *SV_{40} Antiserum.* Heat inactivated rabbit antiserum to SV_{40} strain VA 45-54 GMK 1 with a titer of 1:625 *vs* 1000 TCD_{50} of that strain in grivet monkey kidney cultures was supplied by Dr. Hilleman(1). In neutralization tests equal volumes of 10-fold dilutions of antiserum and 1000 TCD_{50} of virus were maintained at 4°C

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