Aminooxyacetic Acid: Interactions with Gamma-Aminobutyric Acid and the Blood-Brain Barrier

Abstract. *The hypothesis that the administration of aminooxyacetic acid, a competitive inhibitor of aminobutyrate aminotransferase, might allow γ-aminobutyric acid to cross the blood-brain barrier was tested by analyzing the brains of rats for this compound after intraperitoneal injection of aminooxyacetic acid, or a mixture of both acids, and by observing behavioral and physiological changes in cats after similar drug administrations. The results do not support the postulate that γ-aminobutyric acid enters the brain more readily after administration of aminooxyacetic acid.*

Gamma aminobutyric acid (GABA) is a brain metabolite which may play a major role in neural activity. It does not cross the blood-brain barrier to any appreciable extent (*1*). The primary enzyme taking part in its metabolic degradation is aminobutyrate aminotransferase (*2*). Competitive inhibition of this enzyme by aminooxyacetic acid (AOAA) leads to increases in brain GABA for up to 24 hours (*3*). In addition, it recently has been postulated that AOAA might allow systemically administered GABA to cross the blood-brain barrier (*4*). Histological staining had shown that the transferase is localized along the brain-blood vessels and choroid plexus. Studies on mice and rabbits indicated that the systemic administration of both AOAA and GABA produced different and more marked effects than the former alone. We now report on a search for significant behavioral, physiological, or biochemical differences after the injection of AOAA alone or with GABA.

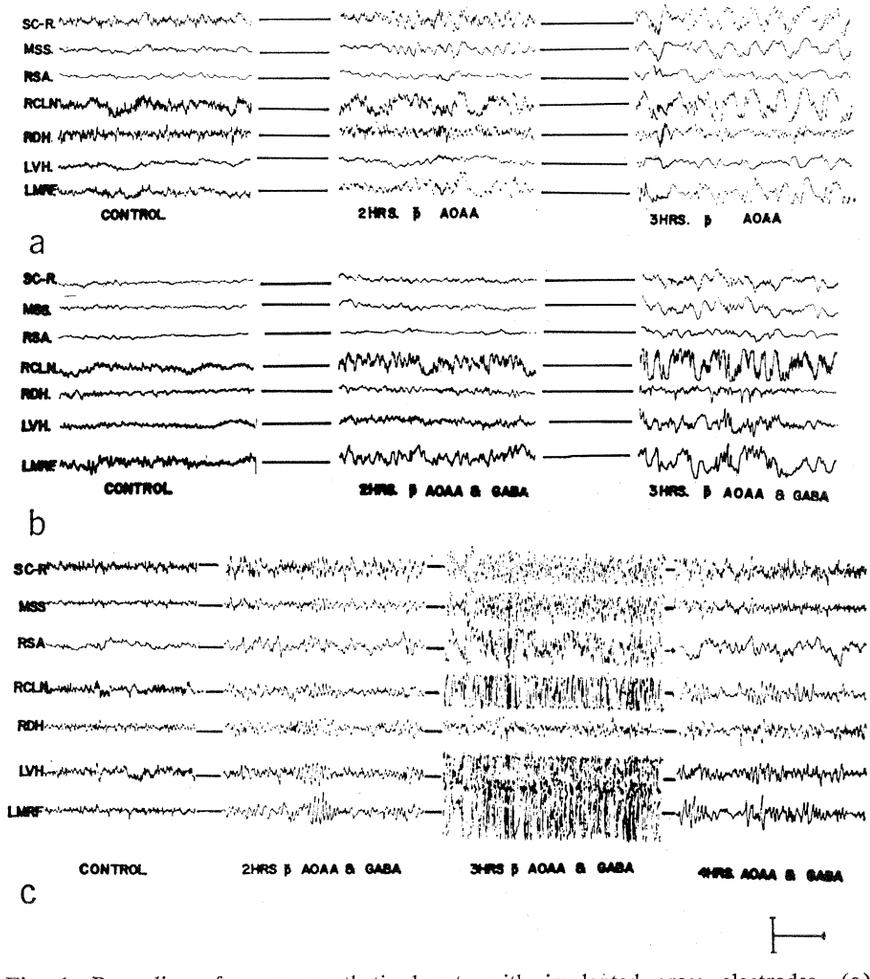

Fig. 1. Recordings from unanesthetized cats with implanted gross electrodes. (a) Control record and activity after the injection of 10 mg of AOAA per kilogram of body weight intraperitoneally at the times indicated; (b, c) the same after 10 mg of AOAA plus 50 mg of GABA per kilogram of body weight, intraperitoneally. There is basic similarity between the hypersynchronous patterns obtained in (a) and (b) despite the difference in drug administration. (c) Pattern with more prominent spiking in contrast with (b) *SC-R*, primary sensory cortex to bone above the frontal sinus; *MSS*, middle supresylvian gyrus; *RSA*, right septal area; *RCLN*, right central lateral nucleus of the thalamus; *RDH*, right dorsal hippocampus; *LVH*, left ventral hippocampus; *LMRF*, left mesencephalic reticular formation. Horizontal calibration, 1 sec; vertical calibration, 100 μv.

Ten intact, unanesthetized cats were injected intraperitoneally either with 10 mg of AOAA per kilogram of body weight or with 10 mg of AOAA and 50 or 100 mg of GABA per kilogram of body weight. The animals were randomly selected, and the conditions were kept as similar as possible during the experiments. All five animals given 10 mg of AOAA per kilogram of body weight vomited during the 1st hour after injection; by 2 hours, all showed mydriasis, ataxia, and the effects of sedation. In those five cats injected with both acids, however, only one vomited, and only two showed the other signs seen with AOAA alone.

Cortical and depth electrodes were implanted into the brains of two cats for long-term measurements. Fifteen experiments were performed during which the animals were unanesthetized and could move freely. The cats were observed, and gross electrical activity was recorded. The experiments were continued for at least 4 hours after each injection. The patterns of vomiting, mydriasis, ataxia, and sedation were similar to those of the intact cats. Salivation was also observed in two of the six experiments in which 10 mg of AOAA per kilogram of body weight was given intraperitoneally alone, and jerking movements or seizures, including one grand mal seizure, were observed in all six cases. Neither salivation or jerking movements occurred when GABA (50 or 100 mg/kg) was administered along with the AOAA.

With the prepared animals the gross electrical changes resulting from injection of AOAA and AOAA plus GABA were as follows. Administration of 10 mg of AOAA intraperitoneally led to the development of a hypersynchronous electrical pattern. These patterns varied from a close resemblance to those associated with an animal in a drowsy state (high-amplitude slow waves were prominent) to activity more characteristic of a seizure with spikes and sharp waves. Although within each experi-

ment one type of pattern tended to predominate, a continuum between these types of electrical activity was indicated. Electrical changes were seen for periods as long as 24 hours and as short as 5 hours, with maximum effects usually occurring from 3 to 4 hours after injection. No clear differentiation could be made between the gross electrical changes seen with 10 mg of AOAA and 10 mg of AOAA plus 50 to 100 mg of GABA per kilogram of body weight; similar patterns in a similar degree were observed in both cases (Fig. 1). There was an indication, however, that GABA might be having an influence, largely moderating, on the changes caused by AOAA. Sinusoidal cortical theta waves were observed during the 1st hour in 50 percent of the experiments with AOAA alone, but were never seen when the combination was administered. Although in approximately an equal number of both types of experiments periods of prominent spiking in all leads were seen, it was only after AOAA alone was injected that a grand mal pattern was observed. Finally, if the grand mal pattern represented the greatest deviation from normal, the least change was observed in an experiment in which 100 mg of GABA per kilogram of body weight was injected along with the AOAA.

There seemed to be poor correlation between the gross electrical activity and the concomitant behavioral and physiological signs. The maximum "drowsy" pattern, for example, often developed at a time when the animal was no longer under sedation and seemed normally responsive to the environment. Usually, moreover, during the first few hours after drug administration, the hypersynchronous patterns could be converted to flatter, more normal ones for several seconds to several minutes by sensory arousal, that is, by clapping. This was not true later in the experiments.

A series of 19 experiments was performed on cats immobilized with *d*-tubocurarine and killed at the end of the experiments. Gross- and microelectrode recordings were made from the primary sensory cortex (posterior sigmoid gyrus). The microelectrodes had tip diameters of about 10 to 20 μ; groups of units rather than individual cells were therefore recorded. The microelectrodes were placed about 1.5 mm below the cortical surface and within 1 mm of the gross surface electrode. Ten milligrams of AOAA or 10 mg of AOAA plus 50 or 100 mg of GABA per kilogram of body weight both produced periods of prominent seizures. The spiking and sharp waves normally appeared within an hour after the injections; the duration of the seizures varied from a few minutes up to 2½ hours. Accompanying any significant period of seizure activity, the unit firing usually followed a regular sequence in relation to the gross activity. As the seizure progressed, the unit increasingly appeared in synchrony with the hypersynchronous waves. At the maximum, few or no units would be seen in the periods of relative electrical silence between seizure bursts and would appear only in synchrony with the highest amplitude waves. With time the seizure activity would abate, and normal cortical and unit activity would return. In two of the six cases in which AOAA (10 mg/kg) was injected alone, unit firing ceased altogether during the seizure period and did not return (Fig. 2). This was never seen when 50 or 100 mg of GABA per kilogram of body weight was administered along with the AOAA. The loss of units did not correlate with any unusual severity or duration of the seizure pattern.

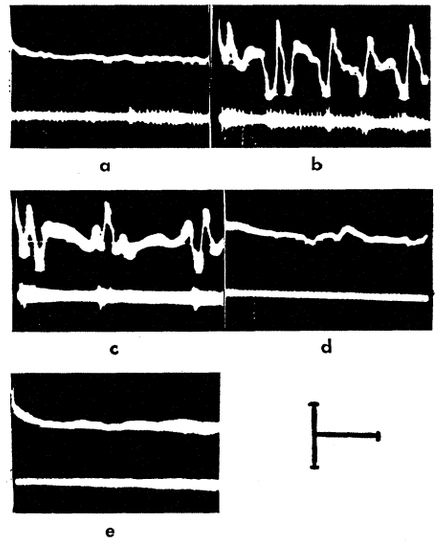

Fig. 2. Records from a short-term experiment with an immobilized cat; control and recordings after administration of 10 mg of AOAA intraperitoneally at the times indicated. The upper channel is a gross recording from the posterior signoid gyrus. The lower channel shows groups of units from the posterior signoid gyrus 1.5 mm below the cortical surface and 1 mm lateral to the gross electrode. In comparison to the control (a), (b) shows spiking and increase in unit activity. In (c), the units appear only in synchrony with the high amplitude spikes; in (d) the loss of unit activity is indicated, and in (e) there is the return to control gross activity with continued absence of units. Horizontal calibration 0.4 second; vertical calibration, 0.8 volt upper channel, 2 volts lower channel.

Injections of 50 or 100 mg of GABA per kilogram of body weight intraperitoneally alone had no discernible effect in any of the preparations used.

Attempts were made to determine the effects of GABA injections at intervals after the administration of AOAA. Since the effects of AOAA injections are variable, this data proved difficult to interpret. No striking change, however, either in behavior or electrical activity was observed when GABA was injected between 30 minutes and 5 hours after AOAA.

Thirty rats were injected intraperitoneally with either saline, GABA alone (100 mg/kg), AOAA alone (25 mg/kg), AOAA and GABA together, or GABA administered 30 minutes after the AOAA. The animals were killed 3 hours after injection. The brains were not frozen *in situ* but were removed rapidly within 2 minutes of decapitation. Free and bound GABA were extracted (*5*) and assayed (*6*). The enzymes used were obtained from Worthington (*7*). In all cases, there was a significant ($p < .05$; Mann-Whitney U test) elevation of free and bound GABA with administration of either AOAA alone or AOAA plus GABA. There was no significant difference, however, between the elevation observed after AOAA alone and that seen with AOAA plus GABA, whether administered simultaneously or with a 30-minute delay.

Contrary to van Gelder's findings with mice and rabbits, in cats GABA plus AOAA apparently does not lead to an accentuation of the physiological and behavioral effects of AOAA, nor are changes observed different from those seen with AOAA alone. If anything, GABA seems to modulate the actions of AOAA. This modulation could conceivably be due to alterations in the blood-brain barrier to GABA. Such an explanation, however, is unlikely. There is no positive evidence to support such a contention, our biochemical data from rats is strong argument against it, and mechanisms other than changes in the blood-brain barrier can be offered to explain the findings. For example, the changes seen with AOAA plus GABA in comparison to AOAA alone were essentially quantitative, not qualitative. The interaction between AOAA and GABA with aminobutyrate aminotransferase is

competitive; aminobutyrate aminotransferase is concentrated along the brain blood vessels (*4*). The simultaneous administration of GABA and AOAA could mean that less AOAA is reacting with GABA-T than when AOAA is administered alone. In essence, we believe that there is some interaction between peripherally administered AOAA and GABA, but probably it is not due to alterations in the blood-brain barrier to GABA.

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Gekkonid Lizards: Average Ages Derived from Tail-Loss Data

Abstract. *Information on the rate of tail loss by autotomy, obtained from mark and recapture data, was used to estimate the average ages of adult individuals of the geckos* Gehyra variegata *and* Heteronota binoei*; the ages calculated were 4.4 and 1.9 years, respectively.*

During a study by one of us of the population ecology of two species of gekkonid lizard, *Heteronota binoei* Gray and *Gehyra variegata* (Duméril & Bibron), which live in parts of the same habitat, several lines of evidence pointed to disparity in average longevity of individuals of the two populations. In particular, marking techniques used in conjunction with sampling showed a sharp reduction in the chance of recapturing *H. binoei*, but not *G. variegata*, about 20 months after the first large batch of lizards was marked. Estimates of recruitment of juveniles to the adult stage—considerably greater for *H. binoei* than for *G. variegata*—also suggested that individuals of the latter species lived longer.

Unable to find a method of determining the ages of living individuals after they had matured to maximum size, we explored the alternative of aging the "population" directly. This approach required some ecological event that marked permanently the individuals affected by it and that had a finite probability of occurrence per unit of time. It could then be argued that the proportion of individuals in a population that were unaffected by the event would be related to the rate at which the event occurred and the average age of the individuals observed. If in a population the rate of occurrence was approximately constant and known, the average age of individuals could be estimated.

The tail is lost quite frequently by the process of autotomy by such lizards as Lacertidae, Scincidae, and Gekkonidae; in many species the regrown portion of the tail is readily differentiated from the original by scale differences. Autotomy results from intraspecific and interspecific interactions [for example, combat associated with territorial behavior, and evasion of predators (*1*)], and it seemed possible that in a well-established population the probability of occurrence of such interactions would tend to have a steady average value per unit of time. Seasonal differences in the probabilities, especially in species that hibernate, require that any average value be estimated over a period of at least a year.

Geckos and other lizards that readily shed tails can lose and replace them many times. Each individual fracture [which occurs at a predetermined plane of weakness across a vertebra (*2*)] is proximal to the previous one. The number of times the tail can be autotomized is limited, but in practice it can be great. The past history of a regrown tail cannot be ascertained by examination because, once loss has occurred, one cannot say with certainty whether it was lost once or more times. Consequently, information on the rate of loss of tails can be obtained only from mark-and-recapture data from a population in which individuals can be frequently recaptured.

The use of autotomy to estimate the average age of individuals of *Heteronota binoei* and *Gehyra variegata* was investigated. Collation of observations made on the tail of each individual on first capture showed the proportions with original tails caught in 1963–64 and 1964–65. The two species proved to be similar (Table 1). The comparable decline in tail persistence in the 2nd year may be related to the fact that, for reasons later outlined, only animals first captured as adults were used in the calculations. Efficiency of capture was high for lizards of all ages, so that little recruitment to the adult population of other than marked hatchlings could occur. In such circumstances the samples of adults used in the 2nd year should be approximately 1 year older than in the first.

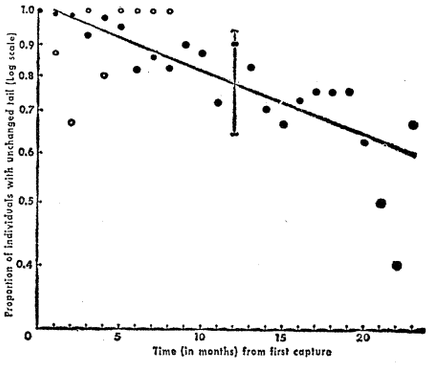

Fig. 1. Rate of tail autotomy in *G. variegata*.

Little difference between the proportions of original tails in the two sexes was recorded, the percentages being:

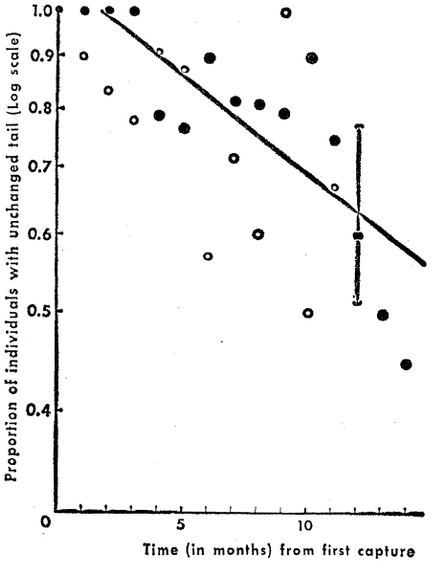

Fig. 2. Rate of tail autotomy in *H. binoei*.