Formation of Ammonium Ion in the Cerebrum in Fluoroacetate Poisoning

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Administration of a fluoroacetate is known to bring about an inhibition of citrate oxidation, thus blocking the tricarboxylic acid cycle and resulting in the accumulation of citrate in various tissues in vivo (1). Hence it is pertinent to inquire concerning the source or sources of the 4-carbon precursors of the accumulating citrate. One obvious possibility is that an amino acid such as glutamic or aspartic could enter the cycle after removal of the amino group. In such a case the nitrogen might appear as ammonium ion, it might combine with glutamic acid to form glutamine, or it might be transferred to some available keto acid by transamination. Brain tissue is known to contain a considerable quantity of free glutamic acid (2).

Fluoroacetate poisoning is characterized by violent epileptiform seizures in some species (3). It is known that convulsions can also be induced by injection of large doses of ammonium salts (4, 5), and that formation of ammonium ion occurs in the brain of the rat on electrical stimulation and during seizures induced by picrotoxin or pentamethylentetrazol (6, 7). Richter and Dawson (6) suggested that the ammonium ion might be the cause of the increased irritability manifested by the convulsions.

These considerations prompted a study of ammonium ion and glutamine in the cerebrum during the course of fluoroacetate poisoning in dogs.

METHODS

The experiments were done on morphinized dogs, except where otherwise indicated. Methyl fluoroacetate was given in doses of 1 mg/kg. The electrical activity of the cortex was recorded. The brain was frozen in situ at the chosen time, and tissue from the cerebrum was taken for analysis. The details of the preparation and a description of the manifestations of fluoroacetate poisoning are given in the preceding paper (8).

In three experiments the fluoroacetate was omitted and seizures were induced by intravenous injection of pentamethylentetrazol (50 mg/kg). In two further experiments the animal was anesthetized with pentobarbital sodium (approximately 40 mg/kg by intravenous injection), the morphine being omitted. Pentamethylentetrazol was given (50 mg/kg intravenously), and the brain was frozen shortly thereafter.

The specimens of frozen tissue were ground and extracted with 10% trichloroacetic acid. Ammonia and glutamine were determined by the Conway microdiffusion technique, essentially as described by Richter and Dawson (6).

The effects of infusion of ammonium chloride solutions and of application of ammonium salts to the cerebral cortex were also studied in morphinized dogs.

RESULTS

In five control animals the ammonium content of the cerebral tissue was found to be 0.17-0.25, average 0.20 µM/gm. The average is not far from that of 0.16 µM/gm reported by Richter and Dawson for whole brains of rats frozen in liquid air (6).

Fluoroacetate. A great increase of ammonium ion concentration in the cerebrum was found to occur during the development of fluoroacetate poisoning (fig. 1). The increase was detectable 35 minutes after the injection of fluoroacetate, and the maximum level appears to have been reached at about 60 minutes. The convulsions also began at about 60 minutes. During seizures the average ammonium concentration was 1.34 µM/gm, representing an increase of almost 7-fold over the control level.

In the preconvulsive group the appearance
of symptoms did not show any close relation to the ammonium levels. In some instances initial symptoms were present with relatively low ammonium values; in others the symptoms were absent with higher ammonium values. In the convulsive group the levels showed no relation to the duration of seizures, which varied from a 17-second period of convulsive activity to 24 minutes of intermittent seizures.

In figure 1 the ammonium values are plotted together with the citrate levels (8) obtained in the same animals. It appears that the release of ammonia precedes the increase in citrate, and on a molar basis the amount of ammonium ion accumulating is usually slightly in excess of the amount of citrate.

In two experiments the ammonium content of the blood was measured on samples taken from the saphenous vein while the brain was being frozen. The blood levels were found to be only slightly elevated, indicating that the ammonia found in the brain is of cerebral origin.

The glutamine values in control and fluoroacetate-poisoned animals are given in table 1. The control values are comparable to those reported by others for brain tissue of various species (2). The averages for the preconvulsive and convulsive groups do not differ significantly from the normal group (P > 0.1 in each case), and no trend upward or downward with time is apparent.

**Pentamethylentetrazol.** In order to compare the ammonium levels in fluoroacetate poisoning with those occurring in seizures induced by other means, three values were obtained during seizures induced by pentamethylentetrazol (table 2). The ammonium levels were found to be considerably above normal, but were lower than those reached during the latter part of the preconvulsive period in dogs given methyl fluorooacetate. In two further experiments, pentamethylentetrazol was given to dogs under pentobarbital anesthesia. In one of these animals the seizure was prevented by the anesthetic, although the electrical activity of the cortex showed an increase in frequency. The ammonium level was only slightly increased (table 2). In the other, mild convulsive activity was present in the cortex for a brief period. The seizure subsided, with occasional slow waves persisting at the time the brain was frozen. In this experiment the ammonium value was in the normal range.

Pentamethylentetrazol did not cause significant changes in the glutamine content of the cerebral tissue.

**Ammonium Chloride Infusion.** It is obvious that the ammonium ion released in the brain during fluoroacetate poisoning might be the cause of the epileptiform seizures which occur. While this possibility has not yet been adequately investigated, five experiments have been carried out in an attempt to determine whether similar convulsions can be induced by gradually increasing the ammonium ion concentration in the brain. Ammonium

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* After injection of methyl fluoroacetate.
chloride was given by intravenous infusion. It was necessary to choose a rate which would surmount the removal of ammonia from the blood by the liver, and to add sodium bicarbonate to the infusion fluid to prevent the development of acidosis. The arterial blood pressure tended to increase during the procedure, and cardiac irregularities were noted.

The infusion brought about a gradual depression of amplitude in the electrocorticogram, a change which is not paralleled during the preconvulsive stage of fluoroacetate poisoning (8). In two experiments convulsive seizures occurred after infusion periods of 9 and 12 minutes, with ammonium levels in the cerebrum approximately twice as high as those attained during fluoroacetate poisoning (table 3). The rate of increase of the ammonium concentration was thus considerably greater than occurs in fluoroacetate poisoning. Likewise ammonium citrate (1 M, neutralized with sodium hydroxide) depressed the amplitude and frequency on subdural injection or when applied by means of saturated filter paper. The ammonium salts did not cause local excitation. In some instances the irritability of the areas was checked by application of 10% pentamethyltetrazol, which induced typical convulsive spikes. Application of 0.2% methyl fluoroacetate to the cortex induced local excitation after a 55-minute period.

**DISCUSSION**

The precursor of the ammonia which appears in the brain in fluoroacetate poisoning cannot yet be designated. The hypothesis that glutamic or aspartic acid enters the tricarboxylic acid cycle when the levels of 4- and 5-carbon acids are diminished, thus releasing ammonia, would appear to require that the blocking of the cycle and the beginning of citrate accumulation precede the release of ammonia. The surprising observation that the accumulation of ammonium ion precedes that of citrate makes it difficult to retain this hypothesis.

It may be noted that ammonium is released from an unknown source in brain tissue post-mortem (6, 10). This ammonium does not appear to come from amide nitrogen. The values in table 1 suggest that in fluoroacetate poisoning, likewise, glutamine is not the precursor. Since hydrolysis of adenosine triphosphate with subsequent deamination has been suggested as a source of ammonia, it should be mentioned that there is little or
no breakdown of cerebral adenosine triphosphate in fluoroacetate poisoning until the approach of the terminal stage (8).

It is notable that the ammonium ion, once released in the brain, does not appear to escape rapidly into the blood stream nor is it quickly converted to glutamate. The occurrence of high ammonium levels during fluoroacetate poisoning and during infusion of ammonium chloride, together with normal glutamine values, appears to cast doubt on the supposed protective function of the glutamic acid-glutamine system (5, 10).

The observed increase of the ammonium level in the brain during seizures induced by pentamethylentetrazol is in harmony with the findings of Richter and Dawson (6) in rats given picrotoxin, and with those of Torda (7) in rats given pentamethylentetrazol. Since the levels found during pentamethylentetrazol seizures were considerably lower than those in fluoroacetate-treated animals which were not having seizures (preconvulsive group), the pentamethylentetrazol seizures can scarcely be attributed to the presence of excess ammonium in the brain. The data therefore support the conclusion of Torda (7) that in pentamethylentetrazol seizures the increase in ammonium ion in the brain is a result of the excitation, rather than its cause.

The significance of ammonium ion as a factor in the initiation of fluoroacetate seizures cannot yet be assessed. The experiments of Torda (7), those of Jenney and Pfeiffer (11), and two experiments reported in this paper have demonstrated convulsive discharges from the cortex after injection of ammonium chloride, leaving no doubt that this substance can induce cortical seizures under certain conditions and at cerebral ammonium levels approximately double those attained in fluoroacetate poisoning. How much of the ammonium remained extracellular in the infusion experiments can only be conjectured. Caution is inspired by the absence of seizures in some of the infusion experiments and the failure of subdurally injected ammonium chloride to induce cortical excitation, but it appears probable that the increased ammonium concentration occurring in the brain during fluoroacetate poisoning is at least one important factor contributing to the occurrence of the seizures.

**SUMMARY**

The concentration of ammonium ion in cerebral tissue of dogs was found to be increased nearly 7-fold during the course of fluoroacetate poisoning. The increase was detectable 35 minutes after injection of methyl fluoroacetate. It reached a maximum at about 60 minutes, and epileptiform seizures supervened at approximately the same time. The increase in ammonium concentration preceded that of citrate and was slightly greater on a molar basis. The cerebral origin of the ammonium ion was indicated by the relatively low levels in the blood. The precursor of the ammonia is unknown. Glutamic and aspartic acids are considered as possible precursors, while neither glutamine nor adenosine triphosphate appears to play such a role.

Ammonium ion released in the brain does not escape into the blood stream at a rapid rate, nor is it quickly converted to glutamic.

The cerebral ammonium concentration was found to be increased during seizures induced by pentamethylentetrazol, but the levels attained were lower than those reached in fluoroacetate poisoned animals during the preconvulsive stage. The release of ammonia during pentamethylentetrazol seizures appears to be a result of the excitation, rather than its cause.

The possible importance of excess ammonium ion in the initiation of fluoroacetate poisoning cannot yet be assessed.
seizures is discussed. Infusion of ammonium chloride induced cortical seizures in two animals and cortical depression without seizures in three animals. Subdural injection of ammonium salts did not induce excitation of the cortex.

REFERENCES