Cerebral Acetylcholine, Serotonin, and Norepinephrine in Acute Ammonia Intoxication[1] (35336)

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(Introduced by R. M. Des Prez)

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Ammonia is currently considered the most likely "toxin" responsible for the induction of hepatic coma (1). The intracerebral mechanism of hepatic coma or even of acute experimental ammonia intoxication is uncertain. One leading possibility, however, is that these conditions are due to the depletion of cerebral acetylcholine (ACh) (1).

The evidence in favor of the ACh depletion hypothesis is as follows: (i) Ammonia *in vitro* interferes with cerebral ACh synthesis (2) and prevents ACh uptake by synaptic vesicles (3); (ii) adenosine triphosphate (ATP) and Acetyl CoA, which may be decreased in brain of ammonia-intoxicated animals (1, 4) are necessary for the synthesis of ACh; (iii) a decreased concentration of ACh has been reported in whole brain of rats intoxicated with ammonia (5); and (iv) ACh is believed to be an important synaptic transmitter in brain (6) and hence may be required for normal cerebral function. On the other hand, the prior *in vitro* studies have employed much higher concentrations of ammonia than are detected in brain of ammonia-intoxicated animals, the *in vivo* studies were carried out in convulsing animals and ACh has been measurd by somewhat nonspecific bioassays. Accordingly, the primary purpose of this study was to assess the cerebral ACh-depletion hypothesis by correlating various degrees of ammonia intoxication with the ACh concentration in the brain, employing a specific, accurate biochemical assay for ACh.

In addition, serotonin and norepinephrine have been tentatively described as neurotransmitters and have been implicated in the control of arousal level (7). A secondary aim of this study, therefore, was to determine the effect of depressed consciousness due to ammonia intoxication on cerebral serotonin and norepinephrine concentrations.

According to current concepts consciousness may depend on a proper integration of nervous impulses received by the reticular activating system in the brainstem and their subsequent transmission to the cortex (8). In this schema, the brainstem may determine the "off and on" aspects of awareness and cortical function may pertain to the content of consciousness. Because of this cerebral functional heterogeneity, and since prior studies in our laboratory have suggested that the brainstem is especially sensitive to acute ammonia intoxication (1, 4), in this investigation the neurotransmitters were assayed separately in the cortex and the brainstem.

Materials and Methods. Sprague-Dawley female rats, 60–80 g in weight, were given intraperitoneally a single dose (7.9 mmoles/kg) of ammonium acetate (NH_4Ac) in physiologic saline. This dose of NH_4Ac results in rapid and reproducible onset of coma without excessive frequency of convulsions or mortality. This ammonia salt was chosen since it results in only slight alterations in the blood pH of the recipients (4). One group of controls was given sodium acetate (NaAc) in a dose equimolar and equal in volume to the NH_4Ac, while another control group consisted of uninjected rats of the same weight. The NaAc-injected controls were sacrificed at times comparable to the

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experimental group. Both experimental and control animals were decapitated without prior anesthesia and the cerebral cortex and brainstem were dissected by hand and immediately assayed. The anatomical landmarks for these areas have been given in detail previously (9).

Acetylcholine was measured by a modification of the specific and sensitive fluorometric method of Fellman (10). This procedure in our laboratory provides a recovery of 91 ± 5.1% (mean ± SE) with known quantities of ACh added to brain and gives a mean variation in duplicates of ±4.1%. Preliminary experiments showed that assay of the fresh unfrozen brain gave comparable values to those obtained with rapidly-frozen tissue. Eserine premedication (to decrease cerebral ACh utilization) was not employed in this study since this substance induces sedation in control rats (9). Serotonin in brain was determined by the procedure of Snyder *et al.* (11) and norepinephrine by the method of Crout (12). Preliminary control *in vitro* experiments indicated that the quantity of ammonia present in brain of the NH_4Ac-intoxicated animals (4) does not interfere with the assays employed. The data are expressed in terms of tissue wet weight since prior studies have shown that brain water content is unaltered in acute NH_4Ac intoxication (4).

Statistical analysis was carried out by the nonparametric Mann Whitney U test (13).

Results. Rats given the single injection of NH_4Ac (7.9 mmoles/kg, intraperitoneally) developed drowsiness in 4–6 min (precoma) which progressed to deep coma by about 10 min. The coma lasted 10 to 20 min and, in the absence of convulsions, resulted in complete recovery. Convulsions almost invariably led to death. Occasional animals which did not develop drowsiness or those which convulsed after the injection of NH_4Ac were not studied. During precoma, the rats readily moved about on tactile stimulation, while in deep coma they only responded to noise, with twitching or convulsions. Both control groups, the uninjected rats and those given NaAc, were asymptomatic.

Table I shows that the cerebral ACh concentration in rats with NH_4Ac-induced precoma and coma remained similar to values noted in the asymptomatic, NaAc-injected controls ($p>0.05$). These comparable values in the two groups of animals were obtained both in the cerebral cortex and brainstem. The ACh values in both groups (NH_4Ac and NaAc) were slightly higher than those found in uninjected rats but the differences were not significant ($p>0.05$). The apparent slight increase in brain ACh in the NH_4Ac- and NaAc-injected rats may be due to the availability of the acetate which may serve as a precursor of ACh via the formation of Acetyl CoA. The higher ACh concentration in the brainstem than the cortex in all animal groups agrees with prior observations (9).

The effects of acute ammonia intoxication on cerebral serotonin and norepinephrine concentrations are shown in Tables II and III, respectively. During precoma the serotonin and norepinephrine levels in brain were normal and this held true also for norepinephrine during established coma. Cerebral serotonin, by contrast, fell modestly ($p<0.05$) in both cortex and brainstem of rats with NH_4Ac-induced deep coma.

Discussion. The present study clearly demonstrates that rats acutely intoxicated

TABLE I. Cerebral Acetylcholine in Acute Experimental Ammonia Intoxication.

		Cortex[a]	Brainstem[a]
Normal control	(10)	5.94 ± 0.89	13.52 ± 0.77
Precoma	NH_4Ac (10)	8.08 ± 0.62	15.55 ± 0.65
	NaAc control (10)	9.62 ± 1.47	16.54 ± 1.14
Coma	NH_4Ac (5)	7.86 ± 0.47	17.37 ± 1.98
	NaAc control (5)	7.31 ± 0.34	13.79 ± 1.43

[a] All values: mμmoles/g of wet wt. The data are presented as means ± SE of the number of animals given in parentheses. Statistical analysis is given in the text.

TABLE II. Cerebral Serotonin in Acute Experimental Ammonia Intoxication.

		Cortex[a]	Brainstem[a]
Normal control	(6)	0.374 ± 0.01	0.829 ± 0.02
Precoma	NH_4Ac (6)	0.360 ± 0.01	0.815 ± 0.02
	NaAc control (6)	0.352 ± 0.01	0.844 ± 0.01
Coma	NH_4Ac (5)	0.284[b] ± 0.01	0.718[b] ± 0.02
	NaAc control (5)	0.379 ± 0.01	0.844 ± 0.03

[a] All values: μg/g of wet wt. The data are presented as means ± SE of the number of animals given in parentheses.

[b] Significantly below control values ($p < 0.05$).

with ammonia have a normal cerebral ACh concentration. This conclusion seems particularly valid since the ACh assay employed is both accurate and specific and the ACh measurements were carried out in those areas of the brain (cortex and brainstem) which are believed to participate primarily in the maintenance of consciousness. These data therefore do not support the ACh-depletion hypothesis as a cause of acute ammonia intoxication, a concept based on prior *in vitro* studies which employed unphysiologically high ammonia concentrations and on data obtained exclusively from convulsing animals (2, 5). Our data are also consistent with recent observations from this laboratory (1) that ammonia does not interfere with pyruvate decarboxylation in the brain and therefore should not induce a depletion of Acetyl CoA, the precursor of ACh. Furthermore, the calculations of McIllwain (14) also suggest that the degree of ATP depletion recently observed in the brainstem of ammonia-intoxicated rats (4) is insufficient to impair cerebral ACh synthesis.

Since the present investigation provides data only about the net available ACh in brain during ammonia intoxication, and it has been estimated that the whole brain may renew its ACh store every 15 sec (15), it is evident that future *in vivo* studies of cerebral regional ACh turnover in ammonia intoxication will be needed. Such investigations await the development of the appropriate microtechniques. Since, however, there is no evidence that ACh utilization is increased in ammonia intoxication and the present study documents normal levels of ACh both during and after the development of NH_4Ac-induced stupor, it seems unlikely that cerebral ACh synthesis is decreased significantly in this condition. Future studies, however, should be carried out to assess whether these data with unfractionated brain ACh also reflect its concentration in subcellular cerebral pools.

Finally, it is evident from this study that alterations in cerebral serotonin and norepinephrine are not responsible for experimental ammonia-induced stupor. Although there was a modest fall in cerebral serotonin with NH_4Ac administration, this occurred only after the coma was established and a similar degree of cerebral serotonin depletion with drugs which impair serotonin synthesis does

TABLE III. Cerebral Norepinephrine in Acute Experimental Ammonia Intoxication.

		Cortex[a]	Brainstem[a]
Normal control	(10)	0.17 ± 0.01	0.53 ± 0.01
Precoma	NH_4Ac (8)	0.19 ± 0.01	0.51 ± 0.01
	NaAc control (8)	0.20 ± 0.01	0.56 ± 0.01
Coma	NH_4Ac (8)	0.18 ± 0.01	0.50 ± 0.02
	NaAc control (8)	0.21 ± 0.01	0.52 ± 0.02

[a] All values: μg/g of wet wt. The data are presented as means ± SE of the number of animals given in parentheses. Statistical analysis is given in the text.

not result in stupor (16). In retrospect, the lack of a significant alteration in cerebral serotonin and norepinephrine during the development of NH_4Ac-precoma is not surprising since precoma occurs within 5 min and serotonin and norepinephrine turnover times in normal brain are of the order of 1 hr (17, 18). On the other hand, the decrease in brain serotonin concentration with ammonia in established coma is of interest since such a change does not occur with ether or cyclopropane-induced anesthesia (17, unpublished data). It may be pertinent, therefore, to examine cerebral serotonin turnover in more prolonged human hepatic coma. Such studies may now be carried out by assaying cerebrospinal fluid 5-hydroxyindoleacetic acid formation after preventing the egress of this substance from spinal fluid by the administration of probenecid (20, 21).

Summary. The primary purpose of this study was to assess the hypothesis that a depletion of cerebral acetylcholine (ACh) may be responsible for the coma induced with ammonia. An ancillary aim was to determine the effect of ammonia on cerebral levels of two other likely neurotransmitters, serotonin and norepinephrine. Acetylcholine, serotonin, and norepinephrine were measured in the brain of rats with NH_4Ac-induced stupor and in asympotomatic NaAc-injected and uninjected controls. Studies were carried out during and after the development of stupor; and both cortex and brainstem were assayed separately since these areas are believed to be primarily responsible for the maintenance of consciousness. Acetylcholine was measured by a specific and accurate fluorometric procedure. In rats with ammonia-induced precoma and coma, the ACh and norepinephrine levels in the cerebral cortex and brainstem were comparable to values seen in control animals. Cerebral serotonin likewise was normal during the development of stupor but fell modestly in both cortex and brainstem of animals with established coma. This study indicates that cerebral regional stores of ACh, serotonin, and norepinephrine are normal during the development of acute ammonia-induced coma and that other mechanisms for this neurologic dysfunction will have to be considered.

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