Effect of Halothane Anesthesia on Regional Acetylcholine Levels in the Rat Brain

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Acetylcholine levels were determined in various regions of the brain of rats subjected to different levels of halothane anesthesia. Increases in regional ACH correlate with neurophysiological depression assumed to be present in these regions at different levels of anesthesia. Non-utilization is a likely cause for the increases in ACH observed.

Crossland and Merrick in 1954 confirmed earlier reports that total acetylcholine (ACH) levels in rat brain increased during anesthesia, and noted that deepening anesthesia resulted in even higher levels of ACH. ACH in the brain is of interest because it is currently viewed as one of the neurotransmitters in brain. The opposite of Crossland and Merrick’s finding has also been shown: over-activity depletes the brain of ACH, e.g., convulsing animals exhibit lower brain ACH levels. Thus it appears that the amount of ACH found in brain correlates with the state of activity.

Many investigators have shown, however, that all anesthetics do not affect the brain in the same fashion. The essence of these studies is that anesthetics affect different cerebral structures in a different manner and at different concentrations. For example, the reticular formation is affected more profoundly than other areas, at light levels of anesthesia. The net result of these effects seems to be a selective depression of the central nervous system, inhibiting some functions such as pain perception and avoidance behavior, but leaving others such as functions of the lower brain stem, relatively intact. If changes in ACH can be considered a general reflection of central nervous system activity, it should be possible to demonstrate changes in regional brain ACH parallel to the degree of neurophysiological depression. Such correlation would provide evidence that increases in ACH reflect reduced utilization. The objective of this study was to discover if such correlations do in fact exist.

Methods

In each experiment 3 rats (Sprague-Dawley strain) from the same shipment and cage were used. One rat was designated as a “control” and sacrificed. A second animal was anesthetized with halothane (Fluotec vaporizer 5 liters/oxygen/minute) in a bell jar, quickly tracheotomized after induction and connected to a Harvard rodent respirator incorporating a non-rebreathing system. Succinylcholine 1-2 mg. was administered intraperitoneally to facilitate artificial respiration (succinylcholine, systemically administered, did not interfere with assay of cerebral ACH as determined in preliminary experiments). Respiratory was maintained at a tidal volume of 3 ml. and rate of 100/minute. The concentration of halothane was reduced to 0.5 per cent, bitemporal electroencephalographic leads were inserted and the electroencephalogram (EEG) was monitored. Light anesthesia was defined as rapid low voltage activity on the EEG; this level was held for 10 minutes after which the animal was killed (fig. 1). A third rat was subjected to the same procedure, except that

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Fig. 1. Upper trace is the ECG taken at low paper speed. Lower trace is the EEG. Recording I shows the tracings of a lightly anesthetized animal recording II, a deeply anesthetized animal. Recording speed 15 cm./minute. The heavy bars represent time intervals of 12 seconds.

Deep anesthesia was maintained with 2–3 percent halothane, as indicated on the EEG by slow voltage activity with burst suppression (fig. 1). After 10 minutes at this level the animal was sacrificed.

The animals were killed by immersion in liquid nitrogen for 10 seconds, which rapidly brings the brain temperature to near freezing levels. After freezing the animals were decapitated, the heads transferred under ice to a cold room (–10° C.) where the brain was removed and dissected rapidly by region as shown in figure 2. The segments of the brain ("cortex," "upper," "middle," and "lower") were immersed in liquid nitrogen immediately after dissection, powdered in a mortar, the powder weighed and transferred to test tubes containing 3 ml. of 5 percent trichloroacetic acid (weight/volume) in ether. The preparation was then homogenized, ether evaporated in a stream of air, the dried homogenate taken up in acidified tyrode (pH 4), centrifuged at 19,000 × g for 20 minutes, washed with acidified tyrode (1 ml.) and recentrifuged at 19,000 × g for 20 minutes. The combined supernatant fractions represented the extract. This extract was kept frozen overnight and after thawing, was neutralized with solid NaHCO₃ to pH 7 and assayed against a standard consisting of hydrolyzed extract (5 minutes boiling at pH 10), to which, after neutralization, ACH was added (a precaution suggested by Feldberg). Assay for ACH was performed on a distal segment of guinea pig ileum using the method described by Barlow, Scott and Stephenson, and by Stephenson with modifications. The

Fig. 2. Schematic drawing of rat brain (A) before dissection and (B) after removal of cortex and cerebellum. The lines indicate I) "upper brain stem the region rostral to the superior colliculi; II) "middle brain stem" the region below the corpora quadrigemina; III) "lower brain stem," the region caudal to the inferior colliculi.
modifications were: the kymograph lever in our experiments had an amplification of 1:8, the tissue bath a volume of 5 ml. The extracts and standards were added by means of tuberculin syringe at 90 second intervals.

Each extract was assayed 3 times against a higher and lower standard in a partial Latin square design of ABC, BAC, CBA; B being the unknown, A and C the known standards. Calculation of the amount of ACH present in the extract was done by averaging the height of contractions and plotting the average of the unknown against the standards on semilog paper. The quantity found was expressed in nanomoles (millimicromoles) of ACH-base, per gram of wet tissue.

**Results**

Nineteen separate experiments were performed. The control levels found are given in Table 1. The control levels found in our experiments compare well with ACH levels found by Crossland and Merrick, with the exception of the middle and lower brain stem which were analyzed separately by us but combined by Crossland and Merrick; our values were higher.

<table>
<thead>
<tr>
<th>Cortex</th>
<th>Upper brain stem</th>
<th>Middle brain stem</th>
<th>Lower brain stem</th>
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<tbody>
<tr>
<td>14.91 ± 1.99*</td>
<td>30.78 ± 1.64</td>
<td>30.57 ± 1.78</td>
<td>24.28 ± 1.98</td>
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* Standard error of the mean.

Table 2 shows the changes in ACH observed between the “control” and “light” anesthesia group and the “light” and “deep” group of animals.

**Discussion**

The data show that increases in the level of ACH vary between regions as well as with depth of anesthesia. The increase in ACH, as shown by Crossland and Merrick, was not time dependent. The cortex demonstrated the largest increases in ACH on an overall percentage basis. The observation that whole brain increases in ACH content with anesthesia is probably the result of increase of ACH in the cortex since the cortex represent almost half of the brain weight. In order to correlate the significant increase in ACH content observed under light anesthesia with neurophysiological phenomena the observation of Liddell and Phillips is pertinent that anesthesia affects the apparent size of cortical motor areas stimulated as determined by single pulse stimulation of the cortex of the monkey. Deepening the level of anesthesia by intermittent injection of anesthetic either reduced the apparent size of the cortical motor area from which the authors were able to elicit specific motor responses or entirely eliminated motor responses to cortical stimulation. It is conceivable that decrease in response is the result of reduced release of ACH thus leading to the increased ACH levels as found in our experiments.

The “upper brain stem,” which included the basal ganglia in our section, showed a significant increase in ACH only during deep anesthesia. Catecholamines and 5-hydroxytryptamine are generally considered the predominant neurohumors in this region, although the role of ACH in this part of the brain has not been established, Shute and Lewis demonstrated many cholinergic nerve structures by means of histochemical techniques and McLennan demonstrated release of ACH from the caudate nucleus.

The middle brain stem incorporating the areas beneath the anterior and posterior colliculi, showed a marked rise in ACH under light anesthesia and no further increase under deep anesthesia. Such changes agree with the
observations of French, Verzeano and Ma-
goun,\textsuperscript{18} as well as others,\textsuperscript{19} describing depression of the reticular activating system during light anesthesia. That part of the reticular forma-
tion concerned with regulation of sleep and arousal is present in this region of rat brain.\textsuperscript{20} The present finding of an increase in content of ACH fits the concept of depression of activity leading to decreased usage of transmitter.

The lower brain stem remained unchanged in content of ACH during light and deep anesthesia. This can be explained by the fact that functions located here are relatively unaffected by anesthetics, unless toxic doses are adminis-
tered. Regulation of respiratory function is perhaps the only exception.

The experiments described above were conducted with halothane. Other agents might well show differences in the regional distribution of increases of ACH, correlating with different patterns of depression seen with those agents.

Several mechanisms are possible to explain the increase in content of ACH during anesthesia. ACH concentrations in brain may rise as a result of excess ACH production, decreased elimination of ACH or both. Increased production may be the result of activation of the synthetizing enzyme, choline acetylase or increased availability of substrate acetyl-co-

enzyme A. It is difficult to disprove experimentally the activation of choline acetylase because of the problem of assessing enzyme activation \textit{in vivo}. Acetyl-coenzyme A, the precursor of acetylcholine, has not been assayed with success in small amounts of brain tissue; therefore increased substrate availability cannot be shown. However, increased production of ACH seems unlikely on the grounds that a neurohumor necessary for neural activity should not be synthesized at an increased rate under conditions of cellular depression. Reduced hydrolysis of ACH owing to inhibition of the hydrolyzing enzyme acetylcholinesterase has been disproved in the presence of several depressants rendering this unlikely as a cause for the increases in ACH.\textsuperscript{21} Thus the most likely explanation for the findings here is that in the anesthetic state ACH is not released, accumulating in regions which are depressed neurophysiologically.

Summary

Increased levels of ACH correlate with the known alterations of physiologic function of localized areas of brain. The most likely explana-
tion in this phenomenon is that the levels of ACH increase because of decreased utilization of this neurotransmitter.

References

3. Richter, D., and Crossland, J.: Variation in acetylcholine content of the brain with physi-
4. Elliott, K. A. C., Swank, R. L., and Henderson, N.: Effects of anesthetics and convul-
8. Takashishi, R., and Aprison, M. H.: Acetyl-

INFORMED CONSENT The effects of obtaining informed consent upon the results of clinical analgesimetry were studied in two groups of patients receiving morphine sulfate 10 mg. for the management of postoperative pain. Preoperative consent for using an "experimental drug" was obtained from one group but not from the other. A standard patient interview technique was used for the assessment of pain relief. The study indicated that obtaining consent for this type of investigation does not significantly alter the results. There is, however, a tendency with the consent group to respond more favorably to treatment. (DeKornfeld, T. J.: Informed Consent and Clinical Analgesimetry, Fed. Proc. 25: 501 (March) 1966.)

PHEOCHROMOCYTOMA Pheochromocytoma is an uncommon disease accounting for an estimated 0.5 to 0.6 per cent of all cases of hypertension. Pheochromocytomas have occurred with sufficient frequency in association with neurofibromatosis and carcinoma of the thyroid to indicate that these illnesses are related. Surgical removal of the tumor remains the only preventive procedure for a patient with pheochromocytoma. In cases of malignancy or in those rare patients in a debilitated state, contraindicating surgery, long-term management with oral phenoxybenzamine has proved beneficial. Since most untreated patients die of cardiovascular disease rather than their tumor, blockage of the cardiovascular and metabolic effects of circulating norepinephrine and epinephrine may result in survival for many years. The demonstration that these patients may have a low blood volume, and in particular a greatly reduced red cell mass, has proved to be of great importance. This defect may be corrected preoperatively by either transfusion with packed red cells or the control of blood pressure with oral phentolamine or phenoxybenzamine. While no control evaluation has been done on the use of phenoxybenzamine preoperatively, there is a strong impression that this treatment simplifies anesthetic management and greatly reduces the severity of postoperative hypotension and shock. At the time of surgery, phentolamine remains a valuable drug for the control of hypertension. (Crout, J. R.: Pheochromocytoma, Pharmacol. Rev. 18: 651 (March) 1966.)