Morphology of Emetic Chemoreceptor Trigger Zone in Cat Medulla Oblongata. (18670)

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Recent experimental analysis of the nervous regulation of vomiting by Wang and Borison (1) represents a major revision of the traditional concept of the emetic mechanism. Contrary to the prevailing belief that certain emetic agents such as apomorphine and digitalis glycosides induce vomiting by direct excitation of the vomiting center, these workers have demonstrated that the drugs mentioned act on a separate locus situated in the floor of the IVth ventricle and designated by them as the "chemoreceptor trigger zone for emesis"(1,2). They localized this trigger zone in the vicinity of the ala cinerea by studying the effects of chronic lesions placed in the medulla oblongata of dogs with the aid of electrocautery. By trial and error the size of the lesion was reduced to the smallest permissible limits consistent with the elimination of the vomiting induced by centrally-acting emetic drugs. In this manner successful preparations were obtained in which most of the dorsal vagal nuclei remained intact. It was concluded by Wang and Borison that the trigger zone is situated in the dorsolateral portion of the ala cinerea since this portion was consistently destroyed by their operations.

Because of its widespread visceral connections, the ala cinerea has long been suspected of having a role in the regulation of vomiting, and for many years it was actually considered to be the site of the vomiting center(3). This contention proved to be wrong when the vomiting center was shown to be situated in the lateral reticular formation of the medulla (1). Nevertheless, the ala cinerea retained some measure of its original importance with its newly acquired distinction as the probable location of the emetic trigger zone.

Histological examination of the periventricular medullary substance was made by us to characterize that portion of the ala cinerea which purportedly had the unique function of a chemoreceptor emetic trigger zone. No morphologically distinguishable structure could be identified in the dorsolateral portion of the ala cinerea. In the cat, there exists a highly vascular ependymal layer(4) which immediately overlies the dorsal vagal nuclei. This vascular ependymal layer has been signified by many workers(5-8) as the area postrema. In the present report, the term "ala cinerea" will be used only in reference to the grey matter embodying the dorsal vagal nuclei. Close study of the area postrema has revealed along its lateral margin a triangular zone which dips inwards between the ala cinerea and the descending vestibular root. This triangular zone is an essentially non-neural structure which, to the authors' knowledge, has not been previously described. This structure, which is now considered by us to be the chemoreceptor trigger zone, will hereinafter be designated as the CT zone. To examine the hypothesis that the CT zone may be the actual site of attack by central emetic drugs, the experimental study reported below was undertaken.

Experimental. The CT zone comprises less than a cubic millimeter of tissue on each side of the IVth ventricle. Because of the obvious difficulty in making accurate bilateral lesions restricted to such a minute area, localization of the emetic chemoreceptor function was attempted by seeking the common

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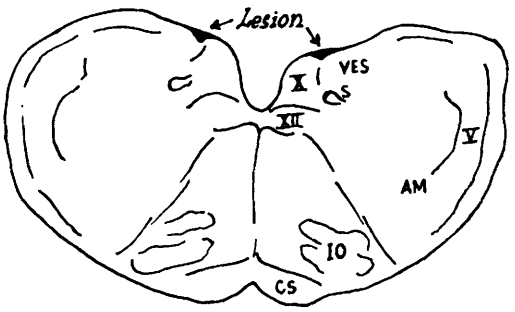

FIG. 1.
Cross-section of cat medulla oblongata above obex. Drawing made from projection of section from operated specimen. Animal died but did not vomit following intravenous ouabain 0.06 mg/kg, 10 days postoperatively. VES, descending vestibular root; S, fasciculus solitarius; AM, nucleus ambiguus; IO, inferior olive; CS, corticospinal tract.

topographical correlate in a series of cat preparations. With the use of electrocautery, lesions were placed in the region of the CT zone in 10 cats, all of which failed to vomit postoperatively to normally effective emetic doses of one or more of the cardiac glycosides. Prior to testing the operated animals, it was established that 0.08 mg/kg lanatoside C, 0.08 mg/kg scillaren A or 0.06 mg/kg ouabain will almost invariably induce emesis in the normal cat within 30 minutes after intravenous injection. Of 15 trials with the above mentioned drugs in the 10 operated cats tested as long as 3 months postoperatively at dose levels at least as high as those indicated, there was only a single vomiting response in one animal; this emesis occurred after an atypical latent period of 8 hours. Four of these cats died within half an hour following injection of the cardiac glycosides, yet no vomiting was elicited. Apomorphine was not used in the present experimental study since in the cat it is more prone to induce fits of hyperexcitability and combativeness than to elicit emesis. Histological study of the brains of 6 of the operated animals, including 3 which died as a direct result of the cardiac glycoside administration, showed that, while there was a variable degree of damage in the ala cinerea, the area postrema and the vestibular root, these structures were largely spared (Fig. 1). The CT zone, however, was found to be destroyed in all 6 brains examined.

Anatomical. In the cat the CT zone is seen in cross section as a more or less triangular structure, the apex of which extends for a short distance into the substance of the medulla between the ala cinerea and the descending vestibular root (Fig. 2). It is contiguous medially with the tissue which forms the main body of the area postrema. The CT zone is approximately one mm in length rostrocaudally. Rostrad, it extends a short distance beyond the rostral limits of the bulk of the area postrema; at this level the triangular configuration is particularly evident since the structure lies in direct relation medially to the dorsal sensory nucleus of the vagus and laterally to the vestibular complex. The caudal end of the CT zone has not been definitely determined but it appears to begin shortly rostral to the caudal edge of the area postrema which becomes evident at the obex.

In all preparations numerous small blood vessels enter the medulla between the CT zone and the vestibular complex or pierce the surface of the zone directly. These vessels give rise to small branches which form an extensive network in the area. The ependymal epithelium exhibits the same morphological modifications in the region of the CT zone as it does over the area postrema(7).

A variety of staining technics applied to the CT zone have revealed that it is essentially a non-neural zone occupied largely by fibroblasts and cells resembling protoplasmic astrocytes and astroblasts. Preparations appropriately stained for microglia, oligodendroglia and fibrous astrocytes revealed few such elements in this area. The vascular feet of the "protoplasmic astrocytes" are especially abundant in the CT zone. These appendages vary in form from thick single podia to rather long complexly branched processes. In addition to the "protoplasmic astrocytes" and the fibroblasts, a few small clusters of irregularly shaped cells with nuclei of similar size and form as those of ependymal cells were found scattered throughout the CT zone. The cytoplasmic bodies of these cells stained deep red in trichrome preparations. Collagen fibers are abundant and are found in close association with the numerous small blood vessels in the area.

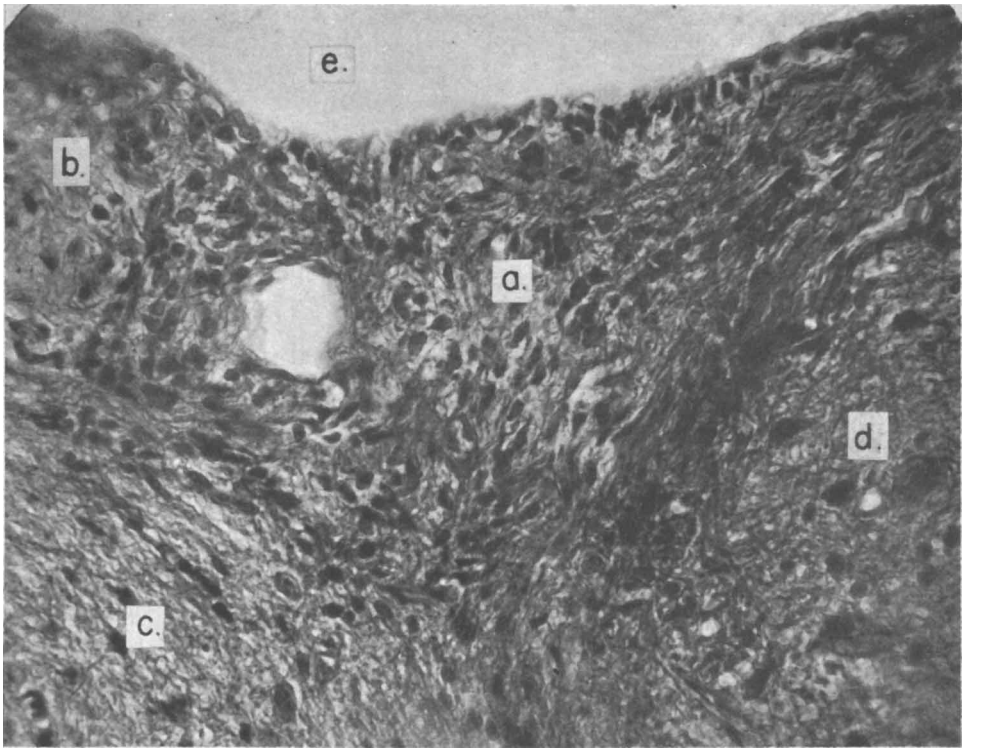

FIG. 2.
Photomicrograph of region of the CT zone. a. CT zone, the triangular area bounded by b, c, and d; b. area postrema proper; e. dorsal sensory vagal nucleus; d. descending vestibular root; e. fourth ventricle. Masson trichrome stain. Magnification approximately 500 diameters.

One silver preparation revealed the presence of fine nerve fibers in the CT zone. Many of these fibers course irregularly through the zone or between this structure and the adjacent vagal and vestibular nuclei. More discrete groups of fibers pass to the apex of the zone and emerge as loose bundles extending deeply towards the nucleus of the fasciculus solitarius. These bundles are joined by similar but smaller groups of fibers from the main body of the area postrema. Many fibers of the CT zone were observed to terminate in fine nerve endings close to the nuclei of the "protoplasmic astrocytes." Others appeared to end "freely" or adjacent to smaller nuclei, the identity of which has not been established.

The area postrema closely resembles the CT zone in its cellular composition but differs in that it generally stains less densely in hematoxylin and eosin and in Masson trichrome preparations and in that it contains scattered small neurons. The present findings, as concern the non-neural elements of the CT zone, are in rather close agreement with those of King's study of the area postrema in the cat(7). However, we have observed a greater abundance of vascular feet in the CT zone than he has described for the closely associated area postrema. We also take exception to King's conclusions in that we found numerous nerve fibers in the area postrema as well as in the CT zone. The directional organization of these fibers was more evident in the CT zone. For a survey of the literature regarding the area postrema, the reader is referred to Cammermeyer(8).

Comment. This communication reports the existence of what is believed to be a hitherto unidentified medullary structure which functions as a central emetic chemoreceptor apparatus. The problem of adventitious interruption of fiber pathways is invariably encountered in experiments involving lesions in the central nervous system. Pinschmidt(9)

has shown that the emesis following parenteral digitoxin in the dog persists after denervation of the carotid and aortic chemoreceptor mechanisms. This finding was confirmed by us in one debuffered cat which gave a characteristic emetic response to lanatoside C injected intravenously. However, this animal failed to vomit after the CT zone was destroyed. Thus, destruction of the CT zone abolishes emesis by eliminating the selective site of attack of the cardiac glycosides and not by interrupting afferent pathways from extramedullary receptors.

The similarity in morphological characteristics between the CT zone and the medially situated area postrema raises the question whether these structures have similar functions. At the present time this question cannot be answered. Nevertheless, the fact remains that of these two regions, the CT zone was the only one found to be consistently destroyed in the operations resulting in abolition of emesis induced by intravenous digitalis. However, this does not exclude the possibility that the CT zone may have an additional role as the final common pathway for the transmission of activity from the contiguous vascular ependymal tissue (area postrema). Experimental study of this problem is underway.

From the anatomical part of this report it is clear that the CT zone borders upon, but is not a part of, the ala cinerea. It becomes questionable whether the ala cinerea is at all involved in the nervous regulation of vomiting. In this connection, there is the possibility of a species difference in medullary morphology between the dog and the cat. The experiments reported by Wang and Borison(1,2) were performed solely in the dog. The findings herein presented were obtained in cats, although some work was also done on dogs. The CT zone is not as readily identified in the dog as in the cat. This cellular formation is far less circumscribed in the dog and it is not improbable that scattered cells subserving the specialized emetic chemoreceptor function may actually exist in the canine ala cinerea. In the cat, however, the CT zone is seen as a clearcut morphological entity quite distinct from the dorsal vagal nuclei.

The discovery of a specialized chemoreceptor zone in the medulla oblongata makes it probable that other such structures exist in the central nervous system and in relation to the ependymal lining of the brain cavities. The demonstration of such areas in relation to the third ventricle, for example, would be of immense value in bridging large gaps in our present knowledge of hypothalamic and hypophyseal physiology. Furthermore, it remains to be determined whether the CT zone functions solely as an emetic chemoreceptor zone or whether it also has other functions analogous to those of the carotid and aortic bodies. In addition, the anatomical proximity of pressoreceptors and chemoreceptors in the carotid and the aortic regions suggests that pressoreceptors may exist in close association with the chemoreceptors of the CT zone. Such strategically located pressoreceptors could explain the emesis caused by elevated cerebrospinal fluid pressure. Further study is in progress to extend the present findings and to investigate other species.

Summary. 1. A hitherto unidentified structure in the medulla oblongata is described. It is an essentially non-neural zone situated between the ala cinerea and the vestibular complex and is contiguous medially with the area postrema which overlies the ala cinerea. The zone consists largely of fibroblasts and cells resembling protoplasmic astrocytes and astroblasts which have an abundance of vascular feet abutting on a dense network of blood vessels. Numerous nerve endings found in this zone are observed to connect with a loose fiber bundle which passes towards the nucleus of the fasciculus solitarius. 2. Experimental studies made with the ablation technic have yielded evidence that this structure is the anatomical framework of the medullary chemoreceptor trigger mechanism for the emetic center. Destruction of this chemoreceptor trigger zone in cats makes the animals refractory to the emetic effect of the intravenously administered cardiac glycosides—

9. Pinschmidt, N. W., PROC. SOC. EXP. BIOL. AND MED., 1946, v61, 7.

lanatoside C, scillaren A and ouabain—in doses which are known to be effective in intact animals.

The authors acknowledge the technical assistance of T. D. Harris. They are also indebted to Professors L. S. Goodman, W. S. Loewe and T. F. Dougherty for advice and encouragement.

Received April 2, 1951. P.S.E.B.M., 1951, v77.

Morphologic Observations by Electron Microscopy of the Leon Strain of Poliomyelitis Virus. (18671)

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Electron microscopy studies conducted on the Brunhilde strain of poliomyelitis virus have been reported(1). The following study was undertaken with the Leon poliomyelitis strain. The Leon strain of poliomyelitis virus, which was isolated in Los Angeles in 1937 by Dr. John F. Kessel, was furnished by Dr. Jonas Salk and Major Byron Bennett of the Poliomyelitis Research Center, Municipal Hospital, University of Pittsburgh, Pittsburgh, Pa. The particular pool used, Leon Gen. XXI, has a 50% end point of $10^{-4.2}$ in rhesus monkeys by intracerebral inoculations.

Procedure. The rhesus monkeys used in the current study were healthy and were negative to the tuberculin test. They weighed approximately 5 lb each. Two monkeys were inoculated intracerebrally with 1.0 cc of a 1/50 dilution of poliomyelitis virus (Leon strain). Typical symptoms of poliomyelitis were noted on the 6th day after inoculation and the monkeys were sacrificed. The spinal cords were removed aseptically and pooled. Two monkeys were inoculated intracerebrally with 1.0 cc of a 1/50 dilution of normal monkey spinal cord which had been removed 8 days earlier, ground, and kept in the deep freeze at −40°C. No symptoms of central nervous system disorder were evidenced and both monkeys were sacrificed the 6th day post inoculation. The spinal cords were removed aseptically and pooled. The infected and normal pools were each ground with alundum and diluted to a 20% suspension with physiological saline. The 2 suspensions were then subjected to 5 minutes centrifugation at 1,000 rpm in an angle centrifuge. The supernatants were removed from the 2 specimens and filtered through a type ST size L3 Seitz filter. The filtrates from the 2 suspensions were then subjected to centrifugation under refrigeration in a Spinco ultracentrifuge for 3½ hours at 50,000 rpm. The temperature of the refrigerated outer jacket stayed constant at −14°C during the centrifuge run. After the 3½ hour centrifugation period, the supernatants from the specimens were discarded, and the sediment from each specimen was resuspended in 1.0 cc of physiological saline. A small drop of each suspension was placed on several film supports. After all excess fluid had been removed with small capillary pipettes, the films were dried and shadowed with chromium(2) at arc tangent 1/6 and examined under the RCA electron microscope, type EMU.

One monkey was inoculated intracerebrally with 0.4 cc of the concentrated normal material, and each of 2 monkeys was inoculated intracerebrally with 0.4 cc of the concentrated infected material. The monkeys receiving the concentrated infected material showed typical poliomyelitis symptoms after a period of 6 days and were sacrificed. Histological sections of the cords and brains showed typical pathological lesions of poliomyelitis. The monkey receiving the concentrated normal monkey cord appeared normal during a 30-day

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