

ELECTROENCEPHALOGRAPHIC FINDINGS IN GUINEA PIG ANAPHYLAXIS

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RECENT attention has been given to the role of the central nervous system of the guinea pig during anaphylaxis. Work has been reported indicating that specific physical changes within the central nervous system of this animal can modify or inhibit the onset of anaphylaxis.

Filipp and Szentivanyi^{1, 2} added impetus to this approach by reporting that bilateral lesions placed in the tuberal region of the hypothalamus protected the guinea pig against experimentally produced anaphylaxis. Karczewski³ described the protective effect of electric stimulation of the brain of guinea pigs during histamine shock. Freedman and Fenichel⁴ published their findings that bilateral lesions in the reticular formation of the midbrain at the level of the superior colliculus inhibited development of anaphylactic shock in sensitized animals.

These published reports raise the question of whether central neural mechanisms exist which can directly influence the extent of the anaphylactic reaction in the shock organ of the guinea pig.

Heimlich, Dunlop, and Smith⁵ followed the above work by taking electroencephalographic recordings of guinea pigs in the hypothalamic and midbrain sites already mentioned in order to determine whether localized electroencephalographic findings occur during anaphylactic shock in these areas. In addition, they recorded electrical activity during anaphylaxis from the superficial cortex as well as from the preoptic area and the dorsal hippocampus. The central nervous system activity during anaphylaxis, histamine injection, and asphyxia was found by these investigators to be characterized by high amplitude spike activity, flattening of background activity, desynchronization, and high-amplitude slow waves. These findings were felt to be related to irritative mechanisms in the central nervous system during anaphylaxis. Since these mechanisms were not localized to specific areas in the central nervous system, they were thought to be the result of a generalized reaction.

Independently of Heimlich's work, the following studies were performed in order to determine whether the electroencephalographic patterns which are characteristic of anaphylaxis would be found in specific areas of the guinea pig brain.

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METHODS

Twenty-one male and female guinea pigs* weighing from 500 to 900 grams were used. Eighteen of these were sensitized by an intraperitoneal injection of 1 ml. of 1:5 dilution of pooled horse serum† on two successive days. They were challenged by the intravenous injection of undiluted horse serum (0.1 ml. per kilogram of body weight) a minimum of 21 days following the initial injection. The remaining three animals were not sensitized and were used as controls. They, too, were challenged with an equal volume of antigen per body weight in the same fashion as the sensitized animals.

The animals were anesthetized by means of veterinary sodium pentobarbital (Nembutal, 60 mg. per milliliter). Satisfactory anesthesia was achieved with doses of 0.55 ml. per kilogram of weight. When necessary, anesthesia was supplemented with ether insufflation. A superficial neck vein was cannulated with a small-caliber plastic catheter through which saline or the test serum was later introduced. A cortical lead was placed on the dura over the frontal lobes, and deep placements of recording stainless steel needles, having diameters ranging from 0.2 to 0.3 mm., were accomplished by means of a Horsley-Clarke stereotaxic instrument. All the animals had leads in or near the dorsal hippocampus, the tuberal region of the hypothalamus cited by Filipp and Szentivanyi, and the reticular formation of the midbrain at the level of the superior colliculus, as mentioned by Freedman and Fenichel. For additional recording sites, some animals had electrodes in the preoptic area, in the reticular area of the midbrain at the level of the inferior colliculus, and/or in more caudal regions. The stereotaxic coordinates for the placements were empirically determined with the aid of a rat atlas. In addition, an electrocardiogram was taken by means of bilateral subcutaneous recording needles inserted over the thoracic cage.

Baseline recordings were made. Saline of a volume equal to the challenging dose of horse serum was introduced into the catheter. This was followed, after return of baseline conditions, by injection of the test dose of the horse serum.

The animals which did not die from anaphylaxis (one of the test group together with three controls) were killed by the injection of sodium pentobarbital (Nembutal). In order to determine the anatomic site of the electrode placements, electrolytic lesions were produced, the brain was removed and fixed, and Weil preparations of the sections were made. A gross examination of the internal organs was made with special note taken of the size and appearance of the lungs. Microscopic sections of the lung, stained with hematoxylin and eosin, were also prepared.

RESULTS

Behavioral Effects.—All but one of the eighteen sensitized animals developed fatal anaphylactic shock following a challenge dose of horse serum. The one animal resistant to shock developed transient behavioral effects. None of the nonsensitized animals used as controls revealed any discernible behavioral reaction to challenging doses of the antigen.

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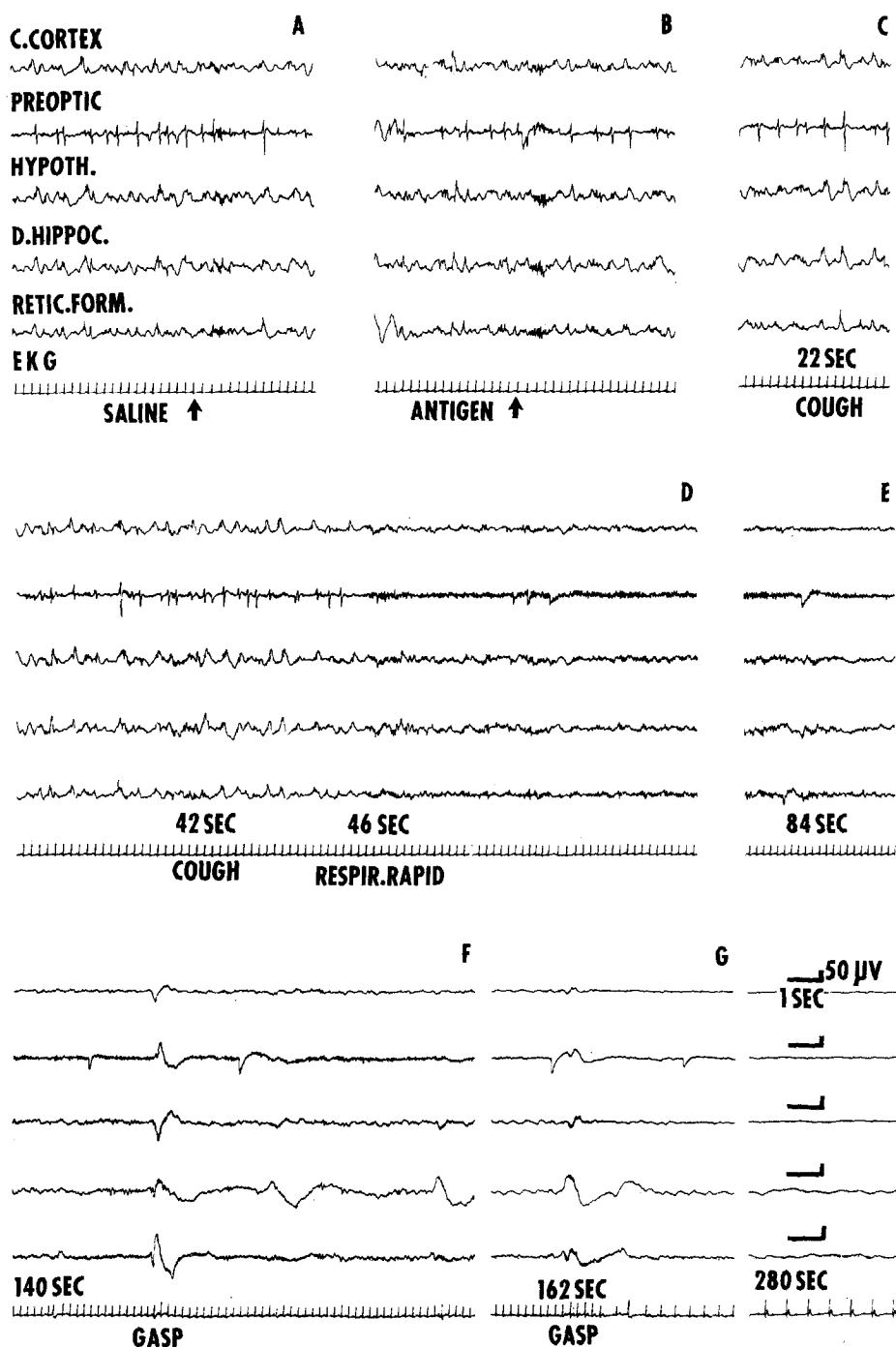


Fig. 1.—Electroencephalogram and electrocardiogram recording of guinea pig No. 21 during anaphylaxis. Arrows indicate time of intravenous injection. Recorded time in seconds refers to time elapsed following intravenous injection of antigen. Onset of behavioral effects is also designated. Representative placements are shown in Fig. 3.

Although the animals were sufficiently anesthetized to permit apparently painless surgical preparation and immobilization on a Horsley-Clarke stereotaxic apparatus, most of the sensitized ones showed a behavioral response in some modified form when subjected to intravenous injections of the antigen. The sensitized animals, in response to the antigen, coughed, sneezed, salivated, snorted, blinked, developed piloerection, and showed signs of respiratory distress by rapid or labored respirations. These findings, however, were not nearly as marked as those that occur in nonanesthetized animals, nor were terminal seizures evident, as is often the case in nonmodified anaphylaxis. The anesthetized animals usually revealed only one or a few of the above findings, in contrast to animals developing anaphylactic shock without benefit of anesthesia. A vivid description of unrestricted anaphylactic shock in guinea pigs has been given by Freedman and Fenichel.⁴

Electroencephalographic Findings.—Individual differences in the electroencephalographic patterns were found with respect to the time of onset and the extent of reactions seen. The electroencephalogram of five of the animals revealed immediate transient occurrences of faster low voltage waves (Fig. 1, *A*), indicative of arousal, lasting from a fraction of a second until 20 seconds (Fig. 2, *A*) following a control intravenous injection of saline. In the latter figure, the effect is particularly characterized by a dropping out of higher voltage slow waves. These findings occurred in all leads. In the sensitized animals electroencephalographic signs of arousal ranging from slight increase of fast waves and decrease of voltage to marked sudden desynchronization (Fig. 1, *D*) occurred from 22 to 228 seconds following injection of the antigen—the most frequent range being from 30 to 50 seconds (Table I). Following this, a gradual reduction in wave voltage occurred until electrical activity was no longer recorded. Brain activity in all animals ceased before cardiac action ended (Fig. 1, last segment). The electroencephalographic records of the nonsensitized animals used as controls did not reveal any of the above findings.

In 11 of the 17 animals that died following anaphylaxis, the electroencephalographic signs of arousal occurred within a 10 second range either preceding or following the first behavior reaction to the antigen (Table I). Behavioral changes were found either to precede or to occur simultaneously with arousal more often than to follow it. The onset of behavioral effects coincided with the first electroencephalographic evidence of arousal in six of the animals. While it was possible in some experiments to have missed the initial appearance of slight behavioral signs, there does not appear to be consistent evidence that the central nervous system changes preceded the peripheral ones.

Electrocardiographic Findings.—The intravenous injection of either saline or serum caused an immediate slowing of the rate, along with a slight transient decrease in voltage (Fig. 1, *A* and *B*). Recovery took place within 1 to 2 seconds following the injection. In 10 of the 17 sensitized animals which developed anaphylactic shock, the rates increased from 12 to 48 beats per minute by the time the initial behavioral symptoms appeared. The remaining animals maintained a stable rate. The usual reaction was one of progressive change of the T waves and ST segment. These generally occurred after the initial signs of

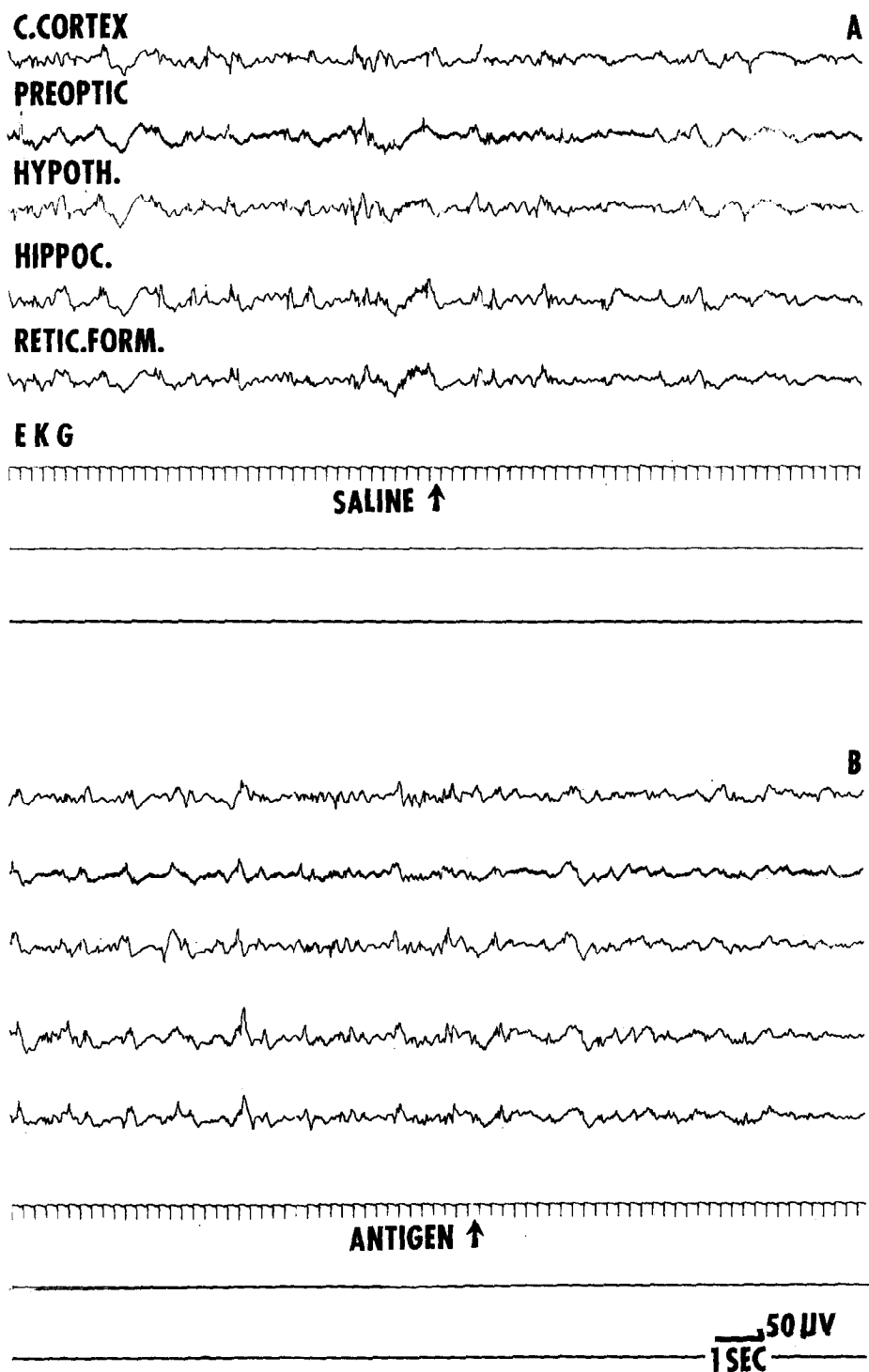


Fig. 2.—Electroencephalogram and electrocardiogram tracings showing arousal pattern in same guinea pig following intravenous injection of saline and antigen. Arrows indicate point of intravenous injection.

TABLE I. TEMPORAL RELATIONSHIP BETWEEN ONSET OF BEHAVIORAL EFFECTS AND ONSET OF ELECTROENCEPHALOGRAPHIC CHANGES FOLLOWING INJECTION OF ANTIGEN

GUINEA PIG NO.	TIME OF BEHAVIORAL EFFECTS (SECONDS)	TIME OF EEG AROUSAL (SECONDS)	TIME OF MAXIMAL EEG DE- PRESSION (SECONDS)	INTERVAL BETWEEN BEHAVIORAL EFFECTS AND EEG AROUSAL (SECONDS)	TERMINAL REACTION
1	38 (blinking)	58	115	> 10	Died
2	57 (respiration labored)	57	85	None	Died
3	52 (blinking)	29	102	> 10	Died
4	47 (cough)	37	48	< 10	Died
5	None noted	30	62	> 10	Died
6	47 (snort)	47	164	None	Died
7*	None present	Not present	Not present	—	Survived
8	37 (respiratory rate increased)	43	75	< 10	Died
9	35 (cough)	27	38	< 10	Died
10	41 (respiratory rate increased)	36	160	< 10	Died
11	40 (respiratory rate increased)	32	80	< 10	Died
12	186 (respiratory rate increased)	228	268	> 10	Died
13	38 (blinking)	38	160	None	Died
14	51 (respiratory rate increased)	51	106	None	Died
15*	None present	Not present	Not present	—	Survived
16	104 (cough)	115	None	> 10	Survived
17	32 (cough)	32	98	None	Died
18*	None present	Not present	Not present	—	Survived
19	125 (blinking)	37	132	> 10	Died
20	22 (cough)	22	194	None	Died
21	22 (cough)	46	140	> 10	Died

*Control animals.

arousal became evident in the electroencephalogram but before the marked depression of electrical activity became apparent. These changes progressed to prolongation of P-R interval and various forms of arrhythmia. In every animal the heart continued beating after respiration ceased and long after any evidence of electrical activity was apparent in the electroencephalograph. The electrocardiograph records of the control animals revealed no changes other than a transient one following the injection of either saline or the antigen.

Autopsy Findings.—In every animal in which anaphylaxis resulted, gross examination of the lungs following death revealed them to be markedly distended and to have a much lighter color than that of the lungs of the control animals. These changes are typical of severe bronchoconstriction in guinea pig anaphylaxis.

The significant finding from microscopic examination of lung sections of animals that died following anaphylaxis was the presence of a traumatic emphysema with marked dilatation of alveoli and a rupture of the alveolar walls. This finding was not present in the lung sections of the control animals.

DISCUSSION

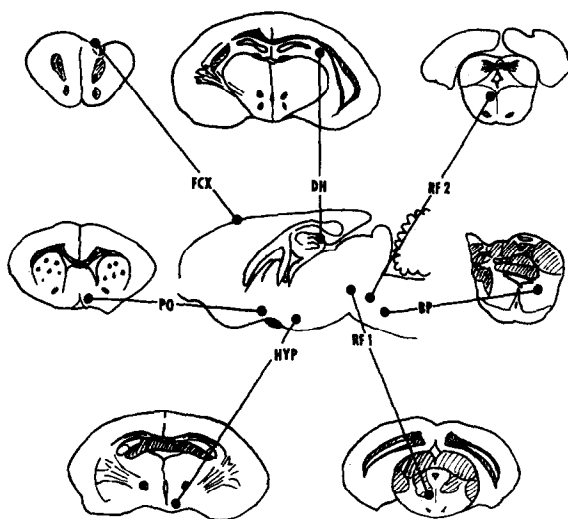
In this series, the predominant electroencephalographic finding accompanying anaphylactic shock was desynchronization, which appeared during light barbiturate anesthesia. This seemed to be related temporally to the behavioral effects (cough, etc.) associated with the anaphylactic reaction. Five of the ani-

mals also exhibited electroencephalographic signs of arousal immediately following intravenous injection of fluid, whether saline or the antigen. This response was the same as the reaction following anaphylaxis and could be differentiated from it only in that it occurred immediately following intravenous injection of fluid and lasted for varying lengths of time up to 20 seconds. The electroencephalographic findings of desynchronization associated with the anaphylactic reaction per se, however, never occurred until at least 22 seconds had elapsed following the challenge with the antigen. Furthermore, the control electroencephalographic pattern did not reappear, the record showing instead a gradual depression of all electrical activity. The findings were not localized in specific anatomic areas of the brain and were present throughout all leads. The electroencephalographic findings of arousal associated with the anaphylactic reaction seem to represent a nonspecific reaction to the peripheral manifestations of anaphylaxis and the resulting anoxia of the brain, just as the arousal resulting from intravenous injection of saline represents a response to the nonspecific effect of intravenous fluid injection.

The above findings substantiate the work of Heimlich and his associates⁵ in that they also interpreted the electroencephalographic findings during anaphylaxis as being related to nonlocalized irritative mechanisms in the central nervous system which probably arose as the result of a generalized reaction. The findings differ, however, from Heimlich's in that his group reported central nervous system activity during anaphylaxis, histamine injection, and asphyxia as being characterized by high amplitude spike activity, flattening of background activity, and desynchronization interspersed with high-amplitude slow waves. Neither high-amplitude spike activity nor high-amplitude slow waves were found in the current study. These variances may be due to differences in the experimental procedures used in the two studies. The Heimlich animals were anesthetized with ether, supported with diphenhydramine (Benadryl), immobilized

Fig. 3.—Schematic representation of sagittal and coronal sections showing anatomic sites used for electroencephalogram recording. Coronal sections were drawn from slide preparations. Electrode placements are marked by solid circles.

FCX—superficial frontal cortex; *PO*—preoptic area; *HYP*—hypothalamic area; *DH*—Dorsal hippocampus; *RF1*—reticular formation of midbrain at level of superior colliculus; *RF2*—reticular formation of midbrain at level of inferior colliculus; *BP*—Lateralmost portion of caudal pons adjacent to brachium pontis.



with D-tubocurarine, and maintained with artificial respiration. In the current study the animals were anesthetized with sodium pentobarbital supplemented with ether insufflation when necessary. This form of anesthesia was selected because it apparently does not interfere with the usual course of the anaphylactic reaction, its central nervous system effect as reflected in the electroencephalogram is known, and it is simple to administer. Although it protected the animal against discomfort and marked motor activity, it did not completely mask all of the behavioral effects occurring during anaphylaxis and did not seem to alter the final respiratory changes. The net result is that the animals in the present study developed both a more intense anaphylaxis as well as a more severe hypoxia, which led to death in a considerably shorter time than in the animals in the Heimlich studies. Their animals experienced a relatively prolonged period of less intense hypoxia and began to display a gradual return towards the control electroencephalographic patterns within 8 to 16 minutes following injection of the antigen. The barbiturate-treated animals usually died within 3 to 4 minutes following the challenge injection, which is approximately the same period of time within which anaphylaxis is fatal in unanesthetized animals. The postmortem gross appearance of the lungs is also similar in both barbitalized and unanesthetized animals developing fatal anaphylaxis. It is possible that the sodium pentobarbital may have masked the high-amplitude spike activity and the high voltage slow waves which were found in their work. It is also possible that the more intense and short-lived hypoxia occurring in the present study may produce different electroencephalographic correlates from those accompanying less intense and more prolonged oxygen deficits. Work remains to be done to determine which of these or other factors may be responsible for the differences in the results.

The present findings also seem to confirm Karczewski's conclusion that the increased electrical activity in both afferent and efferent vagal fibers which he found occurring during anaphylaxis⁶ is of a peripheral and lower central nervous origin rather than higher central. This was based on the finding that increased efferent vagal electrical activity associated with anaphylactic shock was present for at least 10 minutes after the disappearance of all electroencephalographic waves. He believes the increased electrical activity is best explained by stimulation of pulmonary stretch receptors brought into action as the result of the initial bronchoconstriction. He suggests that these afferent impulses elicit further increased bronchoconstriction via a reflex connection with the efferent fibers of the vagus and cites Widdicombe's work^{7, 8} to the effect that stimulation of the efferent vagal fibers can produce bronchoconstriction. It is Karczewski's contention that the activity of this peripheral vagal reflex is one of the mechanisms responsible for the markedly prolonged and irreversible type of bronchoconstriction characteristic of anaphylaxis in guinea pigs. It might prove appropriate to test Karczewski's conclusion further by determining whether bilateral section of the vagus would have a protective effect against anaphylaxis.

Although the work mentioned so far seems to indicate that the central nervous system participates only indirectly in the anaphylactic reaction, at least in the guinea pig, one cannot help but infer that the central nervous system

also contains potentialities for direct control of the reaction. In humans, for example, clinical experience has long shown that emotional factors can either precipitate or intensify asthmatic attacks in predisposed individuals. Knapp⁹ recently reviewed work involving the relationship of emotional factors to bronchial asthma. The work of Noelpp and Noelpp-Eschenhagen¹⁰ might be considered another indication that the central nervous system can participate in anaphylactic reactions. They reported training guinea pigs to develop an asthmatic type of respiration in response to a conditioning stimulus. Ottenberg, Stein, Lewis, and Hamilton¹¹ described "learned asthma" in guinea pigs. Sensitized animals repeatedly developed asthmatic attacks when challenged with an antigen in an experimental chamber. Some of these animals continued to develop respiratory distress when placed in the same chamber even though the antigen was no longer present. After varying exposures to the chamber, devoid of antigen, the reflex became extinct. This led the authors to consider the reaction a learned phenomenon.

The conditioning experiments seem to indicate that the central nervous system in guinea pigs can be directly involved under special conditions in anaphylactic reactions. The experimental design in the present study did not permit the demonstration of primary central nervous system effect in anaphylaxis. Recording of electrical patterns of the central nervous system during the conditioning process, as well as after the reflex has been established, might be helpful in delineating the underlying process.

SUMMARY

1. Electroencephalographic findings during anaphylaxis and resulting asphyxia of anesthetized guinea pigs included an initial period of desynchronization followed by a progressive gradual decrease of electrical activity.

2. These findings were not localized in any specific areas of the brain but were present throughout leads from varied recording sites.

3. The electroencephalographic findings of arousal appeared simultaneously with the first recognition of the overt behavioral response (cough, etc.) of the anaphylactic reaction in six of seventeen animals. In five more animals, the emergence of both behavior effects and signs of arousal occurred within a 10 second period of time.

4. Control intravenous injection of saline produced electroencephalographic signs of arousal which differed from the arousal associated with the anaphylactic reaction per se in its decreased latency, its relative brevity, and its reversibility.

5. It is concluded that the electroencephalographic changes noted arose, initially, as a nonspecific reaction to the peripheral effects of anaphylaxis and, ultimately, as a reaction of the brain to asphyxia.

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