Independence of the Endovestibular Potential in Homeotherms

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ABSTRACT The endolymphatic potential was recorded from various vestibular parts of the labyrinth from which the cochlea (in the case of guinea pigs) or the cochlea, lagena, and sacculus (in the case of pigeons) had been removed. This endovestibular potential of the isolated vestibule declined during anoxia and recovered after anoxia in the same manner as the endovestibular potential of the intact labyrinth. Its non-anoxic level was the same as in the intact labyrinth; *i.e.*, +5 to +8 mv in the pigeon and +2 to +5 mv in the guinea pig. It is, therefore, concluded that the endovestibular potential is independent of the cochlea, stria vascularis, and endocochlear potential.

INTRODUCTION

The endolymphatic potential discovered by Békésy (1) has been studied in both the cochlea (2) and vestibule (3, 4). This potential in the cochlea, the endocochlear potential (ECP), is about 80 mv positive in mammals and about 15 mv positive in birds (5). The potential in the vestibular parts of the labyrinth, the endovestibular potential (EVP), is much lower in homeotherms (4–6).

Three assumptions regarding the EVP are quite common (4, 7, 8):—that nothing compared to the stria vascularis, the probable source of the ECP, is found in the vestibule; that the EVP results merely from spread of the ECP; and that the EVP is therefore of little interest or importance.

These assumptions have very little theoretical or experimental foundation. In fact, the meager evidence available would seem to contradict them. The purpose of this study, therefore, has been to try to determine to what extent, if any, the EVP is dependent upon the ECP.

METHODS

Domestic pigeons were anesthetized with amytal-megimide (140 mg/kg–28 mg/kg) and guinea pigs with dial-urethane (0.5 cc/kg). The trachea was then cannulated. In the case of the guinea pigs, the right bulla and attic were exposed laterally by the standard approach. These were then opened to expose the bony labyrinth. A

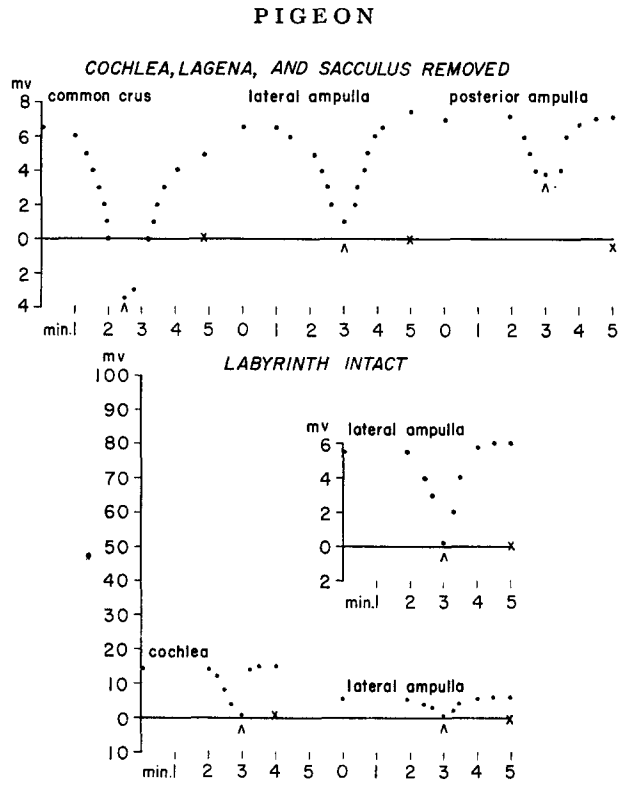

FIGURE 1. Pigeon endovestibular potential in intact and operated labyrinths. Each curve begins (0 min.) at the moment the respirator was stopped. The moment of restarting the respirator is indicated by the arrow at the bottom of each curve. The *x* indicates the level of the final contact reading. In the case of the intact labyrinths, the record of the same ampulla is shown at two different scales. One is for comparison with the endovestibular potentials from the operated labyrinths, and the other is for comparison with the endocochlear potentials of the intact labyrinths. The same scale could not be used for each purpose because of the great difference in level between the mammalian endocochlear potential and the other potentials shown.

small opening was made over the ampulla or part of the scala media to be recorded from. In the pigeon, the right bony labyrinth was exposed laterally behind the tympanum. An opening was then made over an ampulla, the common crus just dorsal to where the lateral and posterior ampullae meet, or at the basal end of the footplate.

In the main series of experiments, the cochlea was removed in order to study the EVP in the isolated vestibule. In the guinea pigs, this was done by breaking open the cochlea and removing the basal part up to the vestibule. Care was taken to remove the basal part of the stria and basilar membrane. In the pigeon, the cochlear cartilage was grasped with forceps and pulled through an opening just basal to the footplate. This removed both the cochlea and lagena. In most cases it was also possible to remove the sacculus with a still finer pair of forceps. In each species, the open-

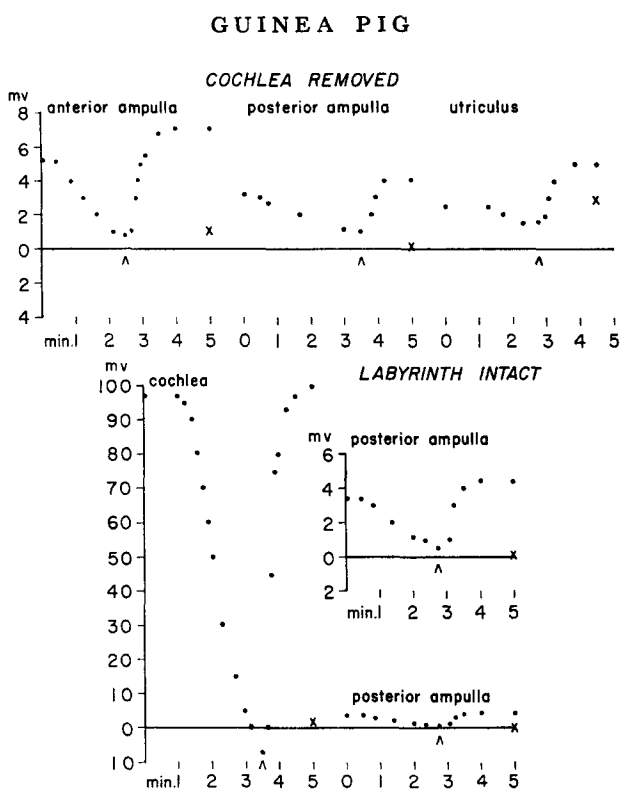

FIGURE 2. Guinea pig endovestibular potential in intact and operated labyrinths. See caption of Fig. 1.

ing to the vestibule was gently closed with bonewax to reduce leakage of fluids and desiccation. The animal was given curare and attached to a respirator. Within 13 to 38 min. after the ablations, the potential in one or more parts of the vestibule was measured. After locating a positive potential, the respirator was turned off and the response of the potential to anoxia observed. After 2 to 4 min. the respirator was again turned on and the response of the potential to recovery from anoxia observed. Since a critical part of the experiment was the effect of recovery from anoxia upon the EVP, it was essential that anoxia be terminated before death of the animal—thus the necessity of using only short periods of anoxia.

The purpose of the second set of experiments was to compare the responses of the EVP and ECP to anoxia. In these animals the labyrinth remained intact, except for the openings for electrode penetration. These animals were also curarized and placed on artificial respiration. The decline during anoxia, recovery from anoxia, and minimum postmortem levels (after lethal anoxia) of the EVP or ECP were then recorded.

Recordings were made with Ringer-filled micropipettes (2 to 4 μ in diameter). The electrodes consisted of two chlorided 100 μ silver wires, one in the pipette and the other in a Ringer-agar bridge (made of a short length of glass tubing) under the

skin in the region of the neck. The DC was measured with a Keithley 200B electrometer. The meter was centered on zero with the pipette tip in the perilymph at the surface of the structure to be penetrated. Advance of the pipette resulted in a negative deflection as the membrane surrounding the labyrinth was penetrated and then a positive potential when the endolymph was reached. Responses of the potentials to anoxia were recorded manually with the aid of a stopwatch. At the end of each experiment a final contact potential (*i.e.* with the electrode again in the surface perilymph) was taken. This final contact potential never differed from the initial contact potential (zero) by more than 4 mv, and the two values were often equal.

RESULTS AND CONCLUSIONS

A total of seven recordings was made from the vestibule of seven guinea pigs (three posterior ampullae, three anterior ampullae, one utriculus) from which the cochlea had been removed. A total of eight recordings was made from the vestibule of seven pigeons (three posterior ampullae, three lateral ampullae, two common crus) from which the cochlea, lagena, and sacculus had been removed. All these potentials declined during anoxia and then rapidly recovered after the respirator was turned on again. Figs. 1 and 2 show curves for three animals of each species. The other recordings were essentially the same as those illustrated. In addition, recordings were made of the EVP of two lateral ampullae and one common crus in two pigeons from which the cochlea and lagena had been removed, but in which removal of the sacculus was not certain. These cases were indistinguishable from those in which removal of the sacculus was certain. It was important, however, to record from the vestibule in the absence of the sacculus as well as the cochlea, since the tegmentum vasculosum (stria) in birds extends up into the sacculus.

It thus seems clear that the EVP not only exists, but that it is quite independent of the cochlea and its ECP. The fact that the EVP and its anoxic modifications can be recorded in the isolated vestibule shows that it is not merely an electrode potential and that it is not immediately dependent upon or produced by the cochlea or stria vascularis. Postanoxic recovery to original levels and maintenance of normal EVP levels during normal respiration (before starting anoxia) show that the EVP recorded in the isolated vestibule was not merely the result of diffusion of a cochlear-derived substance (obtained from the cochlea before its removal) through vestibular membranes of changed permeability due to anoxia.

It is of interest to compare the anoxia-induced responses between the potentials of the different parts of the labyrinth, between the two species, and between the normal and operated labyrinth. It should first be emphasized, however, that detailed comparisons are probably not possible by this method; *i.e.*, by turning a respirator off and on. The responses to anoxia (rate of decline and recovery, delay between change in oxygen supply and response, post-

mortem minimum, rate of return to neutrality from postmortem minimum) must be influenced by the following, and probably other, factors:—the initial composition of the endolymph and perilymph; the permeability of the membranes of the labyrinth; the general health of the animal and its ability to tolerate periods of anoxia; the degree of curarization and whether the animal makes breathing movements after turning off the respirator; the time at which the heart stops beating; the initial level of the potential before anoxia is started; and normal intraspecies variations in labyrinthine physiology. Only the first two factors are really pertinent here. The others merely introduce variability that tends to obscure detailed comparisons.

Within these limits, the EVP of the operated labyrinth is indistinguishable from that of the intact labyrinth. Figs. 1 and 2 show a representative EVP from the intact labyrinth of each species.

During recovery from anoxia, the EVP in the guinea pig almost always returned to levels slightly above those before anoxia (Fig. 2). In the pigeon, however, there is considerable variability so that the level immediately after anoxia may be the same as, slightly above, or slightly below the initial level (Fig. 1). A postanoxic overshoot is also known for mammalian cochlea (9) (Fig. 2).

Since it is fairly well established that the ECP is generated by the stria vascularis (2), it seems reasonable to suppose that the EVP is generated by similar areas in the vestibular parts of the labyrinth. The common assumption that there is nothing in the vestibule comparable to the stria (4, 7) is just not true. Secretory-like areas have long been known in the vestibule of all groups of gnathostomes (10, 11). We have abundant confirmation of this in our own comparative collection of inner ears. It is true that these "secretory" areas are not identical to the stria, but they do show many striking similarities. These areas stain red with eosin and have fan-like radiations at the side of the cell furthest from the endolymph. Electron microscopic studies have shown complex folded membranes at the basal margin of such cells in the stria (12), utriculus (13), and planum semilunatum (14). The little evidence available thus forces one to assume that these areas in the vestibule are at least partially analogous and homologous to the stria.

There are several obvious differences between the EVP and ECP. In each species, the normal positive level of the ECP is much higher than that of the EVP. The 97 mv of the guinea pig in Fig. 2 is somewhat higher than usually recorded. An ECP of less than 60 mv is fairly good evidence of excessive surgical damage or respiratory difficulties. The highest guinea pig EVP recorded (before anoxia) was the 5.2 mv shown in Fig. 2. Most values for the guinea pig vestibule were only 2 to 4 mv. The difference between the positive EVP and ECP in the pigeon is not so great. ECP values are usually between 12 and 16 mv. The highest we have recorded is 20 mv. The pigeon EVP is

higher than that of the guinea pig. It was seldom below 5 mv and was often as high as 8 mv.

The rate of anoxic decline and postanoxic recovery is greater for the ECP than for the EVP in each species (Figs. 1 and 2). The rate of EVP anoxic decline is clearly more abrupt in the pigeon than in the guinea pig, although EVP postanoxic recovery rate seems to be similar in the two species.

The postmortem minimum (*i.e.* the greatest negativity before starting back toward neutrality) was recorded in eleven pigeons and ten guinea pigs. About one-half of these recordings were from the cochlea and the rest from the vestibule. In the guinea pig the ECP minimum (−13 to −39 mv) was always much lower than the EVP minimum (−1.5 to −7 mv). Negative ECP minima of as much as 60 mv have occasionally been recorded by others (9). The initial positive ECP was always 2 to 3.8 times more positive than the postmortem minimum was negative, but the initial positive EVP was actually exceeded by the postmortem minimum (by 1 to 3 mv) in several cases.

In the pigeon, the ECP minimum was also lower than the EVP minimum, but the difference was not as great or as consistent as in the guinea pig. The ECP minimum was from −7 to −19 mv and the EVP minimum (with two exceptions to be mentioned) was from −2 to −7 mv. The initial positive ECP was never more than 2.2 times the postmortem minimum, and in several cases the minimum exceeded the initial positive potential by 4 mv. The initial positive EVP was equal to or as much as four times the postmortem minimum. The small differences between the EVP and ECP postmortem minima may be due partly to the fact that the normal positive potentials do not differ from each other as greatly as in mammals. In two recordings from the common crus, the initial positive EVP was 5.5 mv and the postmortem negative EVP was −13 mv in one case and −25 mv in the other. No explanation is at present available for these exceptionally low negative values. Another common crus potential started at 6.2 mv and fell to only −2 mv.

DISCUSSION

The causes of the difference in level between the normal positive EVP and ECP are not known. A number of hypotheses (by no means mutually exclusive) might be suggested. Perhaps the secretory cells producing the two potentials are physiologically different. The similarities in cytology noted above do not support this. This hypothesis is supported, however, by the fact that the mammalian cochlea has much higher levels of carbonic anhydrase than does the vestibule (15). On the other hand, secretory cells may be very similar but surrounding, functionally related, tissue may be different. For example, the presence of capillaries within the secretory epithelium of the mammalian cochlea but merely beneath the vestibular secretory areas seems likely to be of significance. There may be pertinent differences in the permeability of the non-

secretory membranes of the various parts of the labyrinth. Finally, it is possible that differences in volume and surface-volume relationships might be important.

The causes of the differences in level between the postmortem minimum EVP and ECP are not known either. There could be initial differences in the endolymph composition, although studies of at least some components (3, 16) do not show any great differences. Differences in membrane permeabilities between various parts of the labyrinth could also play a part. The secretory areas in the different parts of the labyrinth may differ in their responses to anoxia, so that some areas remain active longer than others. The fairly great variability of the postmortem minima also remains to be explained.

The presence of an independent EVP presents a number of important but neglected questions. Why is the EVP so much lower than the ECP? If the ECP in mammals is in some way important for the normal function of the cochlear hair cells, would one not expect that the vestibular hair cells would also have found a use for such a high potential? Is the level of the EVP lower because the vestibular hair cells do not need a higher level or because they somehow make more effective use of their endolymphatic potential than do the cochlear hair cells? Would a higher EVP perhaps be of value to the animal, but impossible for some unrecognized restrictions on labyrinthine geometry or on the anatomy and physiology of the stria-like areas? Why are the levels of the EVP so nearly the same in poikilotherms and homeotherms, although this potential is relatively insensitive to anoxia in the first, but remarkably sensitive in the second?

It seems likely that greater attention to the EVP and non-mammalian groups may speed an adequate understanding of the basic biology of the endolymphatic potential.

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